

Developing and validating a new comprehensive glucose-insulin pharmacokinetics and pharmacodynamics model

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Nomenclature

Acronyms and abbreviations

| | |
|-----------|--|
| ACCP/SCCM | American College of Chest Physicians/Society of Critical Care Medicine |
| ADA | American Diabetes Association |
| APACHE | Acute Physiology and Chronic Health Evaluation |
| ARF | Acute Renal Failure |
| ATP | Adenosine Triphosphate |
| AUC/c-ROC | Area Under the Curve |
| BMI | Body Mass Index |
| BSA | Body Surface Area |
| CAVH | Continuous Arteriovenous Haemofiltration |
| CAVHD | Continuous Arteriovenous Haemodialysis |
| CGM | Continuous Glucose Monitoring |
| CI | Confidence Interval |
| CIGMA | Continuous Infusion Glucose Model Assessment |
| CV | Coefficient of Variance |
| CVD | Cardiovascular Disease |
| DISST | Dynamic Insulin Sensitivity and Secretion Test |
| DPP-4 | Dipeptidyl Peptidase-4 |
| ECLIA | Enhanced Chemiluminescence Immunoassay |
| EGP | Endogenous Glucose Production |
| EIC | Euglycaemic CLAMP |
| EN | Enteral |
| FS-IVGTT | Frequently Sampled-Intravenous Glucose Tolerance Test |
| GC | Glycaemic Control |
| GE | Glucose Effectiveness |
| GFR | Glomerular Filtration Rate |
| GIP | Glucose-dependent Insulinotropic Polypeptide |
| GLP-1 | Glucagon Like Peptide-1 |
| HD | Haemodialysis |
| HDL | High Density Lipoprotein |
| HOMA | Homeostasis Model Assessment |
| ICING | Intensive Control Insulin-Nutrition-Glucose |
| ICU | Intensive Care Unit |
| IFG | Impaired Fasting Glucose |
| IGT | Impaired Glucose Tolerance |
| IIM | Iterative Integral Method |
| IQR | Interquartile Range |
| IR | Insulin Resistance |

| | |
|--------|--|
| ISI | Insulin Sensitivity Index |
| IV | Intravenous |
| IVGTT | Intravenous Glucose Tolerance Test |
| MATLAB | Matrix Laboratory |
| MGTT | Meal Glucose Tolerance Test |
| MM | Minimal Model |
| NGT | Normal Glucose Tolerance |
| OGTT | Oral Glucose Tolerance Test |
| PD | Proportional Derivative |
| PK-PD | Pharmacokinetic-Pharmacodynamic |
| PN | Parenteral |
| PS | Polysulfone |
| SI | Insulin Sensitivity |
| SPRINT | Specialized Relative Insulin and Nutrition Titration |
| T2D | Type 2 Diabetes |
| T2DM | Type 2 Diabetes Mellitus |
| TGC | Tight Glycaemic Control |

Abstract

Type 2 diabetes has reached epidemic proportions worldwide. The resulting increase in chronic and costly diabetes related complications has potentially catastrophic implications for healthcare systems, and economics and societies as a whole. One of the key pathological factors leading to type 2 diabetes is insulin resistance (*IR*), which is the reduced or impaired ability of the body to make use of available insulin to maintain safe glucose concentrations in the bloodstream.

It is essential to understand the physiology of glucose and insulin when investigating the underlying factors contributing to chronic diseases such as diabetes and cardiovascular disease. For many years, clinicians and researchers have been working to develop and use model-based methods to increase understanding and aid therapeutic decision support. However, the majority of practicable tests cannot yield more than basic metrics that allow only a threshold-based assessment of the underlying disorder.

This thesis gives an overview on several dynamic model-based methodologies with different clinical applications in assessing glycaemia via measuring effects of treatment or medication on insulin sensitivity. Other tests are clinically focused, designed to screen populations and diagnose or detect the risk of developing diabetes. Thus, it is very important to observe sensitivity metrics in various clinical and research settings.

Interstitial insulin kinetics and their influence on model-based insulin sensitivity observation was analysed using data from the clinical pilot study of the dynamic insulin sensitivity and secretion (DISST) test and the glucose-insulin PK-PD models. From these inputs, a model of interstitial insulin dose-response that best links insulin action in plasma to response in blood glucose levels was developed. The critical parameters influencing interstitial insulin pharmacokinetics (PKs) are saturation in insulin receptor binding (α_G) and the plasma-interstitium diffusion rate (n_I). Population values for these parameters are found to be $[\alpha_G, n_I]=[0.05, 0.055]$.

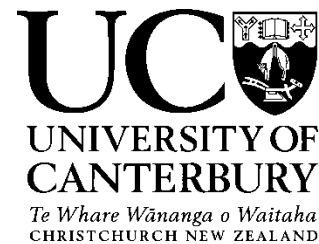
Critically ill patients are regularly fed via constant enteral (EN) nutrition infusions. The impact of incretin effects on endogenous insulin secretion in this cohort remains unclear. It is

hypothesised that the identified S_I would decrease during interruptions of EN and would increase when EN is resumed, where, for short periods around transition, the true patient S_I would be assumed constant. The model-based analysis was able to elucidate incretin effects by tracking the identified model-based insulin sensitivity (S_I) in a cohort of critically ill patients. Thus, changes in model-based S_I given the fixed assumed endogenous secretion by the model would support the presence of an EN-related incretin effect in the population of non-diabetic, critically ill patients studied.

The PD feedback-control model of U_{en} was designed to investigate endogenous insulin secretion amongst subjects with different metabolic states and levels of insulin resistance. The underlying effects that influence insulin secretion i.e. incretin effects were also defined by tracking the control model gain/response and the identified insulin sensitivity (S_I) using intravenous (IV) bolus and oral glucose responses of insulin sensitivity tests. This new PD control model allowed the characterisation of both static (basal) and dynamic insulin responses, which defined the pancreatic β -cell glucose sensitivity parameters. However, incretin effects were unobserved during oral glucose responses as the PD control gains failed to simulate the true endogenous insulin secretion due to potentially inaccurate glucose appearance rates and low data resolution of glucose concentrations.

The net effect of haemodialysis (HD) treatment on glycaemic regulation and insulin sensitivity in a critically ill cohort was investigated. It was hypothesized that the observed S_I would decrease during HD due to enhanced insulin clearance compared to the model, and would be recaptured again when HD is stopped. The changes in model-based S_I metric at HD transitions in a cohort of critically ill patients were evaluated. Significant changes of -29% in model-based S_I was observed during HD therapy. However, there were insignificant changes when HD treatment was ended. Thus, the changes in model-based S_I would thus offer a unique observation on insulin kinetics and action in this population of critically ill patients with ARF that would better inform metabolic care.

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Chapter 1. Introduction

The number of people with diabetes has significantly increased in recent years to approximately 350 million people worldwide (Chen *et al.* 2011). Chronic hyperglycaemia is the main characteristic of diabetes and is directly associated with morbidity and mortality (Capes *et al.* 2000; Krinsley 2003; Krinsley 2004; Van den Berghe *et al.* 2001). In 2004, an estimated 3.4 million people died due to hyperglycaemia (ADA 2006a). Thus, it is suggested that diabetes has reached epidemic proportions, with catastrophic implications on quality of life, healthcare costs and population as a whole (Bonow and Gheorghiade 2004; Chiu *et al.* 2001; King 1999).

The general symptoms of diabetes can be alleviated with glycaemia control protocols in intensive care unit (ICU), which are reported to reduce the risk of other metabolic complications i.e. cardiovascular, sepsis, acute renal failure, and etc. (Chase *et al.* 2008; Evans *et al.* 2011; Krinsley 2004; Van den Berghe *et al.* 2001). Also, understanding the underlying metabolic disorders that contribute to the pathogenesis of diabetes can potentially prevent the risk of developing this disease at the very early stage or provide the best approach to treat diabetes before it becomes chronic. This chapter discusses the overall prevalence, its development and underlying causes of diabetes. Review on its current clinical diagnosis assessment methods and glycaemic control protocols for critically ill patients are also presented.

1.1 Pathogenesis Type II Diabetes Mellitus (T2DM)

The pathogenesis of T2DM is a more gradual process than type 1 diabetes. It generally starts with the pre-diabetes stages of impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), before a clinical classification of diabetes can be made. The progression of this disease is often undiagnosed and untreated for many years, until first health complications start to appear. The physical symptoms that usually occurred in T2DM are listed as follows:

- Dehydration and frequent urination. As blood glucose concentration builds up in the bloodstream, fluid is absorbed from the tissues which cause dehydration. Thus, it increased the fluid consumption and urinates more frequently than normal.

- Increased appetite. Without enough insulin to bind with cells in peripheral tissues and muscles causes energy depletion that triggers intense appetite.
- Weight loss. The body uses alternative fuels stored in muscle and fat as source of energy due to decreased glucose metabolism in the bloodstream.
- Fatigue. The body becomes tired and exhausted as the cells are deprived of glucose.
- Blurred vision. This symptom only occurs when hyperglycaemia causes excessive fluid appearance in the eyes lenses.
- Low immunity system. T2DM affects the body's ability to heal and resist infections. Later, it may cause sepsis if it is untreated.

Typically, T2DM is not recognised early enough to intervene before permanent damage has begun to occur, and is thus often diagnosed only when treating its symptoms or complications at later levels (Gastaldelli *et al.* 2004; Kleinfield 2006). This late diagnosis is due to the nature of the disease development, where noticeable symptoms do not arise until significant irreversible damage has occurred. With accurate early diagnosis pre-diabetic states could potentially identified up to 3-5 years earlier (Andersson *et al.* 2009; Clark *et al.* 2000; Pannala *et al.* 2009) which can significantly reduce the onset of further damage and complications.

T2DM is increasingly diagnosed among children, adolescents and younger adults (Hossain *et al.* 2007). The causes of this epidemic disease are embedded in a very complex group of genetic besides the integrated between quality of life and environmental influences. Given that both insulin secretion and its kinetic are under genetic control, failure of β -cell function and/or IR could theoretically be the primary factors in T2DM (DeFronzo and Ferrannini 1991; Kahn 2003; Poulton *et al.* 2002; Staiger *et al.* 2009; Stumvoll *et al.* 2005). This common aspect presumably reflects the development of IR at the peripheral and receptor level, particularly in the liver, skeletal muscle and heart (Andrews and Walker 1999; Reaven 1988; Shulman 2000).

The chronological treatment of T2DM consists first of lifestyle changes to increase insulin sensitivity. Increases in exercise with healthier diet and weight loss are proven to increase insulin sensitivity and thus reduce the prevalence or impact of T2DM (Duncan *et al.* 2003; McAuley *et al.* 2002; Nishida *et al.* 2004; Tuomilehto *et al.* 2001). This is may be combined with medication,

such as thiazolidinediones (Rosiglitazone), biguanides (Metformin) or sulfonylureas (Gliburide) to enhance insulin sensitivity or stimulate the pancreas secretion (Kahn *et al.* 2006). Lastly, insulin replacement therapy (i.e. exogenous insulin input via insulin pump) as in type 1 diabetes, is required to maintain glucose homeostasis (Hermansen *et al.* 2002; Pickup and Keen 2002; Schaumberg *et al.* 2005; Steil *et al.* 2006)

1.2 Development of model-based S_I test

The model-based insulin sensitivity methods have shown significant ability to diagnose and characterise pre-diabetic state (Beard *et al.* 1986; Bergman *et al.* 1987; Boston *et al.* 2003; Chase *et al.* 2008; Lotz 2007; Mari *et al.* 2001a; McAuley *et al.* 2011; Pacini and Bergman 1986; Wallace and Matthews 2002). Model-based approaches measure the physiological effects that explain the causes to progression of diabetes. Model-based S_I tests typically use empirical methods, mostly regression models that are designed to correlate well with certain gold standard test metrics i.e. euglycaemic/hyperglycaemic clamp (EIC) (Beard *et al.* 1986; Bergman *et al.* 1987; DeFronzo *et al.* 1979; Mari *et al.* 2001a; Pacini and Bergman 1986).

If fasting metrics are used insulin sensitivity is only quantified during a fasting state which may be different to postprandial sensitivity. It is also assumed that the insulin secretion from β -cells can be measured by sampling C-peptide concentrations (Pacini and Mari 2003). The elevated insulin concentrations can cause endogenous glucose production inhibition (EGP). The level of inhibition can be measured with the additional use of glucose tracers (Caumo and Cobelli 1993). Thus, S_I metrics can be different to the real-observed during the dynamic or hyperglycaemic state used in other applications (Scheen *et al.* 1994) as the total S_I is defined as the total amount of insulin sensitivity at peripheral cells and liver.

It is important to model all the kinetic behaviour of insulin and glucose including: insulin clearance, endogenous glucose production, endogenous insulin secretion and to segregate the dynamics (i.e. incretin effects) to fully describe the important aspects of the true metabolic system. Figure 1.1 shows an overview of the physiological effects that can be measured by S_I tests. However, these effects can only be captured by the model depending on the design and application of the tests.

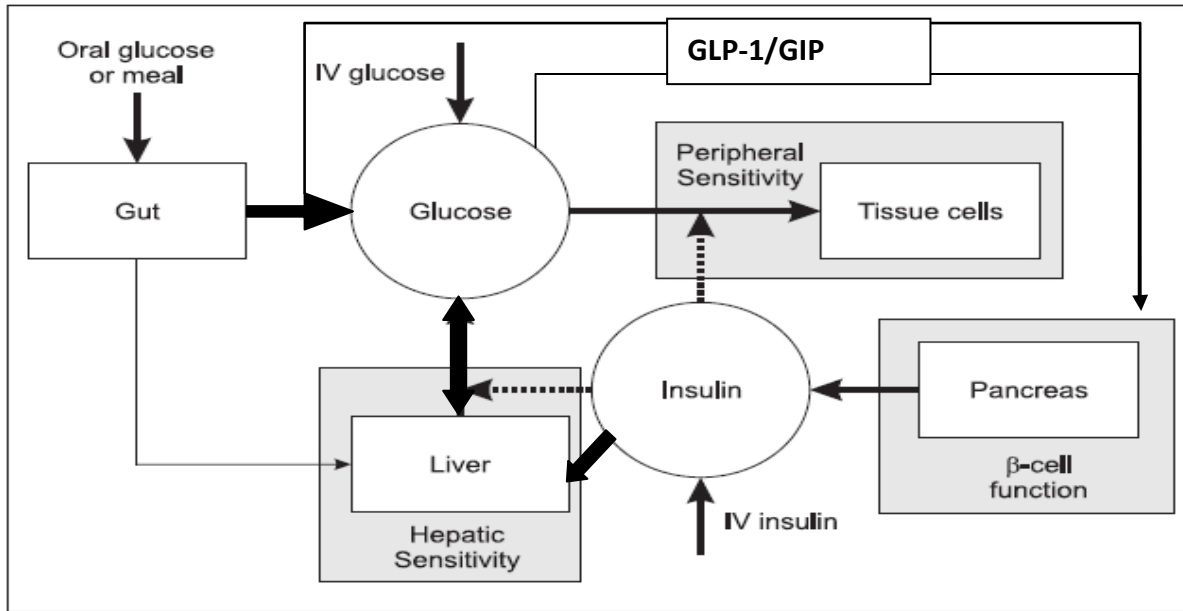


Figure 1.1 Overview of the physiological effects measured by insulin sensitivity tests. Depending on the design of the test, it can measure either one, a combined effect if two, or all three of these effects. The dashed lines indicate a mediated or enhanced effect.

The main effects contributing to insulin dependent glucose uptake (in Figure 1.1) which reflect the insulin sensitivity are the sensitivity of tissue cells to bind insulin (peripheral sensitivity), the effect of insulin on the liver to secrete glucose production (hepatic sensitivity), and the ability of the pancreas to produce insulin with increase in glucose concentration (β -cell function). These effects are time varying and are different in fasting or perturbed states (Scheen *et al.* 1994). Depending on the structural design of the chosen method to assess S_I and its assumptions, one or more of these effects can be combined in the assessment. Thus, different clinical and physiological interpretations can be delivered depending on varying results obtained from the chosen approach.

1.3 Model-based glycaemic control protocol

It is reported that model-based glycaemic control (GC) protocols ensure a reduction in hypoglycaemia in the ICU (Chase *et al.* 2007; Chase *et al.* 2008; Evans *et al.* 2011; Hovorka *et al.* 2007; Le Compte *et al.* 2009). The motivation of these protocols is to reduce clinical burden in ICU and also lessen the chronic outcomes due to organ failure, which increased morbidity and thus mortality. Model-based control relies on a physiological model that captures the glucose-

insulin system dynamics that accurately predict blood glucose, given specific insulin and glucose inputs. A control algorithm can use these predictions to select optimal insulin and nutrition interventions for forthcoming periods.

The potential of models for managing glycaemic levels in critically ill patient is thus becoming realised. However, few models have been clinically validated. For most models, the primary form of validation has been simple fitting of the model to match clinical data (Carson and Cobelli 2001). Occasionally, more rigorous prediction validation, which tests the models ability to predict the outcome of a known intervention on retrospective clinical data (or in a clinical trial) is used. However, only a few clinically validated models can predict within clinically acceptable ranges (Chassin *et al.* 2004; Lotz *et al.* 2008; Pielmeier *et al.* 2010; Plank *et al.* 2006; Wong *et al.* 2006).

Despite its clinical uses, glycaemic control (GC) in ICU also introduced secondary benefits. Studies by Weekers *et al.* (2003) and Langouche *et al.* (2005) indicate that glycaemic control reduces glucotoxicity due to high blood glucose, which in turn reduces oxidative stress and superoxides, which are stress hormone responses that cause damage to the endothelium and vascular walls.

Simple model-based GC protocols have been successfully developed and piloted (Chase *et al.* 2008; Chee *et al.* 2002; Evans *et al.* 2011; Fisk *et al.* 2012; Plank *et al.* 2006; Shaw *et al.* 2006). These model-based methods are able to identify evolving patient-specific parameters and tailor therapy appropriately. The principal of model-based control uses a physiological model that relies on a single, time-varying parameter, in this case S_I , to capture the patient-specific glycaemic response to insulin. As an identified parameter, S_I is prone to capturing other dynamics and metabolic effects which can be used to quantify metabolic dysfunctions and variability in critically ill patients. Although maintaining safe, effective model-based glycaemic control in critically ill patients has proven difficult, due to considerable inter- and intra- patient variability, it may offer the most practical, robust, adaptive and patient-specific solution to manage this issue. Success is thus a function of the model's ability to accurately capture the dynamics of insulin kinetic over time in the highly variable critically ill patient.

1.4 Preface

The objective of this thesis is to understand and validate various pharmacokinetic and pharmacodynamic (PK-PD) models in wider research and clinical settings and present analyses of several important insulin kinetic and metabolic dysfunctions which affect the model-based insulin sensitivity.

This thesis focuses on the kinetic parameters of the (PK-PD) models that affect the insulin secretion, insulin transport kinetics, incretins, and insulin clearance. Additionally, the impact of haemodialysis treatment in critically ill patients and the renal insulin clearance and insulin secretion were also presented. A brief overview of the thesis includes:

Chapter 2 presents the physiology of plasma insulin, glucose, C-peptide and incretins.

Chapter 3 reviews current model-based S_I assessments used in research and clinical settings and its applications.

Chapter 4 investigates the modelling of interstitial insulin actions, using different clinical-validated PK and PD models with DISST data.

Chapter 5 quantify and analyses the incretin effects of critically ill patients that underwent the SPRINT protocol.

Chapter 6 develops a control model for pancreatic insulin secretion as a function of glucose excursions.

Chapter 7 assesses the impact of haemodialysis (HD) therapy on insulin kinetics and action in critically ill patients with acute renal failure.

Chapter 8 and 9 summaries the key aspects of the thesis and present possible future applications for this research.

Chapter 2. Physiology of Plasma Insulin, Glucose, C-peptide and Incretins

This chapter describes the physiology and biochemical characteristics of insulin, glucose, C-peptide and incretin. These effects are captured in dynamic models that seek to segregate the dynamic effects of insulin sensitivity, insulin secretion and other metabolic effects. Hence, it provides foundation of the necessary basic knowledge needed to create effective, realistic models.

2.1 Glucose

Glucose ($C_6H_{12}O_6$) is a monosaccharide used as the main source of energy in the body. It is oxidised in the cells to generate adenosine triphosphate (ATP) molecules which in turn provides energy to the cell (Guyton and Hall 2000). Glucose is transported around the body passively in the bloodstream. It also can be diffusively without insulin taken up by cells in the brain and the central nervous system, as they are highly permeable to glucose. However, muscle, adipose tissue cells and intestinal cells contribute to a majority of the total uptake. If available in abundance, glucose is stored by the liver and peripheral cells as glycogen for future use (Guyton and Hall 2000; Zierler 1999). Most of the body's cells require the hormone insulin to mediate glucose uptake (Despopoulos and Silbernagl 2003; Guyton and Hall 2000). Thus, insulin acts as a biochemical signal that unlocks cellular pathways of cellular glucose uptake, rather than as an integral part of that uptake. Hence, this uptake is referred to as insulin-mediated.

Glycogenesis is the process of storing excess circulating glucose as glycogen in the liver. If glycogen stores are saturated, glucose is converted into fat and stored in the liver and in fat cells in the adipose tissue. These processes can be reversed when the energy demand is high. Glucose is rapidly released from glycogen via the glycogenolysis process if glycogen stores are used, once fat is used via the gluconeogenesis process with amino acids to form glucose (Guyton and Hall 2000; Zierler 1999).

Both glycogenolysis and gluconeogenesis are commonly grouped under and described as endogenous glucose production (EGP) (Zierler 1999). EGP is tightly regulated in the healthy body to maintain basal (minimum) blood glucose concentration. EGP represents net glucose produce by the body, primarily by the liver, and released into the blood (Cherrington 1999). EGP is suppressed when blood glucose concentration is considerably high due to external glucose appearance through meals or to a lesser extent via intravenous bolus (Caumo and Cobelli 1993; Jefferson *et al.* 2001; Pretty 2012). However, low glucose concentrations inverse the process by stimulating glucagon secretion via pancreatic α -cells, which activates glycogenolysis and thus rapidly increases glucose concentrations to prevent hypoglycaemia.

The rate of endogenous glucose production is a function of both stimulus and availability of substrates. In reality, EGP is modulated by the interaction of many hormones in response to metabolic dysfunctions that cause insulin sensitivity irregularities (Gelfand *et al.* 1984; Mizock 2001). As tissue cells fail to respond adequately to insulin, blood glucose concentrations rise. Normally, the liver helps regulate glucose concentrations by reducing glucose production in the presence of insulin. However, this may not occur in T2DM due to insulin resistance that reduced glycogen synthesis and storage and a failure to suppress glucose production. In critical illness, this lack of suppression of EGP is enhanced (Capes *et al.* 2000; McCowen *et al.* 2001; Thorell *et al.* 2004).

The body naturally regulates blood glucose levels as a part of metabolic homeostasis. It is suggested that healthy fasting blood glucose concentration ranges between 4.4-5.1 mmol.L⁻¹ (80-92 mg.dL⁻¹). Prolonged malnutrition or exposure to insulin can result in mild hypoglycaemia (low blood glucose <4.0 mmol.L⁻¹ or 72 mg.dL⁻¹). Severe hypoglycaemia (<2.2 mmol.L⁻¹ or 40 mg.dL⁻¹) can limit the availability of energy to the brain and nervous system that can cause unconsciousness or death. Alternatively, hyperglycaemia is also dangerous and occurs when blood glucose is elevated above safe levels (>11.1 mmol.L⁻¹ or 200 mg.dL⁻¹) with mild hyperglycaemia is defined as BG>7.0 mmol.L⁻¹ (125 mg.dL⁻¹). A subject with a fasting blood glucose range between 5.6 and 7 mmol.L⁻¹ can be diagnosed with impaired glucose tolerance (IGT), while >7 mmol.L⁻¹ (125 mg.dL⁻¹) can diagnose type 2 diabetes. Prolonged hyperglycaemia is highly toxic to a wide range of tissues and can result in diabetic retinopathy leading to partial blindness; and

decay of peripheral capillaries which may finally require body parts be amputated. Importantly, a continuous glucose homeostasis to normal levels is essential for positive on-going health benefits, and is equally true for hyperglycaemic critically ill patients.

2.2 Plasma Insulin

Insulin is a hormone secreted by the pancreas within the β -cells of the islets of Langerhans. Within the islets of Langerhans, β -cells constitute 65-80% of all the cells. Insulin has a leading role in maintaining glucose homeostasis. It enables glucose uptake by muscle and adipose tissue cells, regulates storage and release of glucose in the liver and promotes fat synthesis and storage (Guyton and Hall 2000; Jefferson *et al.* 2001). The pancreas secretes plasma insulin into the portal vein, where it first passes through the liver and subsequently enters systemic circulation. Glucose uptake is activated once plasma insulin is distributed to interstitial fluid, where it binds to cell-membrane receptors (Jefferson *et al.* 2001) as shown in Figure 2.1

Insulin secretion by the pancreas is bi-phasic in healthy subjects (Guyton and Hall 2000; Jefferson *et al.* 2001; Prager *et al.* 1986; Sherwin *et al.* 1974). The first phase is a release of stored insulin in response to significant changes in glucose concentration. The magnitude of the first phase insulin secretion is typically to the rate of changes in glucose and the glucose gradient between the periphery and the portal vein (Cherrington 1999).

The second phase is a prolonged, slow release of newly formed insulin dependent on glucose concentration. When the first phase insulin secretion is diminished, blood glucose concentrations will increase significantly right after oral ingestion. The pancreas compensates for this rise by increasing the second phase insulin secretion, which eventually brings blood glucose concentrations back to normal. However, these high levels of glucose and insulin in the bloodstream may damage the β -cells and further impair their ability to function. As a result, hyperglycaemia and T2DM occur in conjunction with hyperinsulinemia as a result of increased insulin resistance.

This response to glucose can be broadly modeled using a proportional-derivative (PD) feedback controller. This approach has been used to attempt closed loop control of diabetes and for tight glycaemic control (TGC) for critically ill patients (Chase *et al.* 2006; Chee *et al.* 2003; Steil *et al.* 2006). Its popularity in representing insulin secretion is the basis for its use in glycaemic controllers.

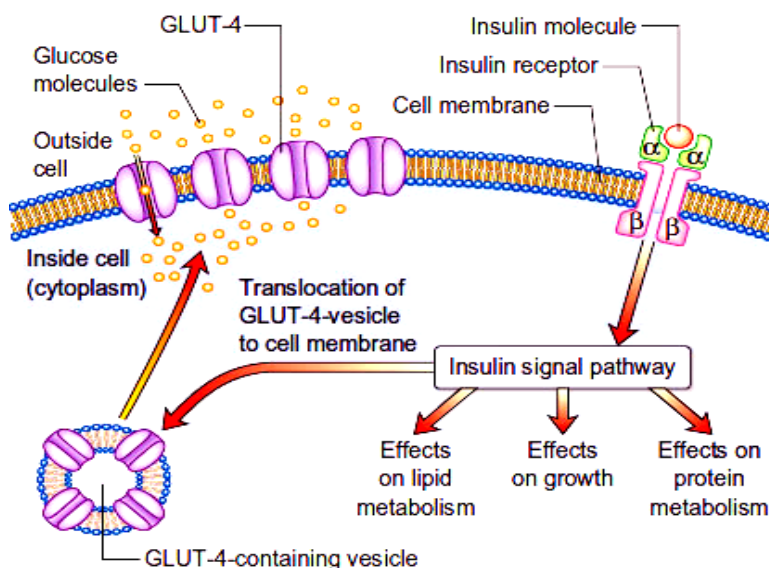


Figure 2.1 Schematic of insulin binding to receptors on tissue cells to activate glucose uptake. (Figure taken from medicinexplained.blogspot.co.nz).

Circulating insulin is mainly cleared through by the liver, accounting for up to 60% of total insulin clearance (Duckworth *et al.* 1988; Ferrannini and Cobelli 1987; Sherwin *et al.* 1974). Approximately 30-60% of endogenous insulin is extracted by the liver in the first pass after it is released into the portal vein (Duckworth *et al.* 1988; Ferrannini and Cobelli 1987; Prager *et al.* 1986; Sherwin *et al.* 1974; Toffolo *et al.* 2006). This mechanism allows a fast response and control insulin circulation and kinetics. Insulin is also cleared by the kidney (Duckworth *et al.* 1998; Mak 1995; Rabkin *et al.* 1984) and through cellular degradation after binding to allow glucose uptake in the periphery (Guyton and Hall 2000; Jefferson *et al.* 2001). Insulin has two different half-lives at 4-6 minutes and 20-30 minutes (Duckworth *et al.* 1998; Turnheim and Waldhausl 1988).

Plasma insulin concentrations control cellular glucose uptake, which consequently affects blood glucose levels. When glucose concentrations are high insulin suppresses the hepatic release of stored glucose (EGP). Insulin also causes hepatic glucose storage through promotion of glycogen synthesis (via glycogenesis) as glucose concentration increases. In summary, insulin is essential in controlling and maintaining glucose levels and homeostasis.

2.3 Plasma C-peptide

Like insulin, C-peptide (connecting peptide) is produced by the β -cells located in the islets of Langerhans in the pancreas (Despopoulos and Silbernagl 2003; Guyton and Hall 2000). C-peptide is distinguished as a by-product of insulin secretion, as both peptides originate from the precursor Proinsulin, which then splits into insulin and C-peptide, as shown in Figure 2.2. Thus, both species are secreted in equimolar amounts (Rubenstein *et al.* 1969). The main difference is that C-peptide is only cleared by the kidney, so it is very stable in use to estimate endogenous insulin secretion (Van Cauter *et al.* 1992).

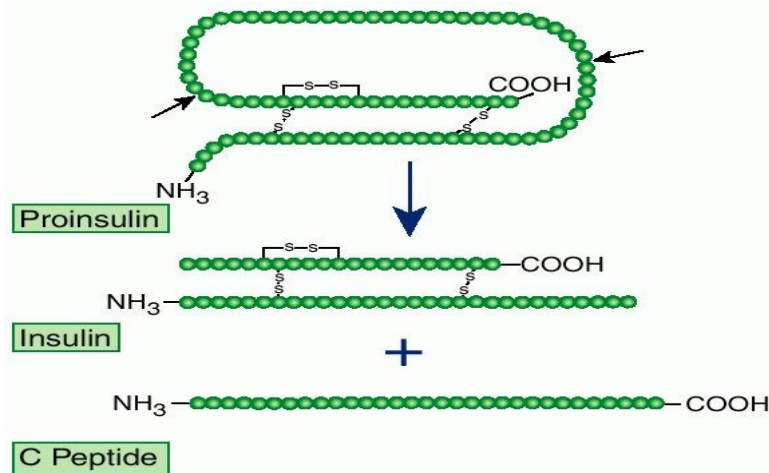


Figure 2.2 Proinsulin chain is joint by two chains of insulin and C-peptide. The precursor proinsulin is cleaved internally at two sides (*arrows*) to yield insulin and C-peptide. (Figure taken from medical-dictionary.thefreedictionary.com/insulin).

Historically, C-peptide was assumed to be a waste product of insulin secretion (Wahren 2004). However, a recent study has indicated a biologically active role, in binding to cell membranes and activating intra-cellular signalling pathways, resulting in improved renal and nerve functions

(Luppi *et al.* 2011). These identified effects are not critical for this research and generally do not affect the modelling approach as its function does not affect its appearance or degradation.

It is suggested that C-peptide is only cleared by the kidney and it thus has a longer single half-life (~2-5 times longer) than plasma insulin (Rubenstein *et al.* 1969). Thus C-peptide has higher concentrations than insulin in the peripheral circulation and less fluctuation in its concentration compared to insulin. These effects allow uncomplicated measurement and models to capture the kinetics of C-peptide, and thus endogenous insulin secretion.

In particular, plasma C-peptide concentrations reveal the pancreatic insulin secretion due to the equimolar secretion of the hormones. In fact, the slower and less variable clearance by a single pathway (rather than three) makes C-peptide modelling more reliable for estimating endogenous insulin secretion rather than using insulin concentration itself. As a result, the models that illustrate C-peptide compartments are independently linking between C-peptide kinetics and insulin kinetics (Eaton *et al.* 1980; Faber *et al.* 1979; Hovorka *et al.* 1996; Lotz 2007; McAuley *et al.* 2011; Polonsky *et al.* 1986; Van Cauter *et al.* 1992).

2.4 Incretins

Incretin effects are defined as insulin responses triggered by a group of gastrointestinal hormones that enhance insulin secretion from the β -cells of the pancreas after glucose ingestion (Creutzfeldt and Ebert 1985; Nauck *et al.* 1986). Incretin hormones cause glucose to produce a greater insulin response than intravenous glucose infusions for the same content of glucose (Polonsky *et al.* 1988). They thus enhance insulin secretion responses. There are two recognised main incretin hormones: glucagon-like peptide 1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP). These hormones are rapidly deactivated by the enzyme dipeptidyl peptidase-4 (DPP-4) as illustrated in Figure 2.3.

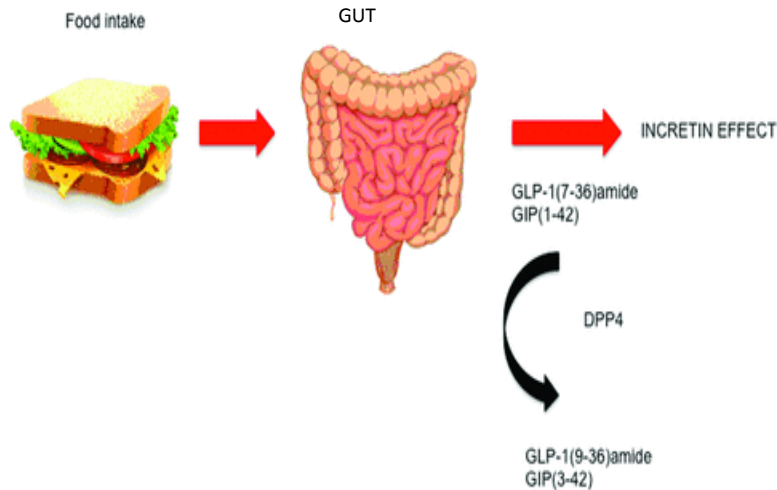


Figure 2.3 Schematic of the incretin effects by meal ingestion (Phillips and Prins, 2012).

Prior experiments show that up to 60% of the insulin responses after oral glucose consumption are not caused by a direct mechanism of glucose with β -cells in the islets of Langerhans, but by the secretion and action of incretins (Nauck *et al.* 1986; Nauck *et al.* 1993). Initially, GIP was identified as the first incretin hormone based on the insulinotropic actions of intestinal mucosal extracts which had been depleted of GIP (Creutzfeldt and Ebert 1985). A continuing incretin effect was noticed when circulating GIP was inactivated by anti-GIP antibodies (Ebert *et al.* 1983). This led to the discovery of additional incretin hormones, GLP-1. This peptide was found to be an insulinotropic agent that responsible for stimulating insulin secretion, especially at increased glucose concentrations (Kreymann *et al.* 1987; Nauck *et al.* 1993; Qualmann *et al.* 1995). Both peptides are suspected to suppress glucagon secretion from the pancreas (even at basal levels) (Gromada *et al.* 1998; Schirra *et al.* 1996) and decelerate gastric emptying (Nauck *et al.* 1997; Ørskov *et al.* 1996) and acid secretion (Schjoldager *et al.* 1989), and thus achieve their effect on insulin secretion.

2.5 Summary

To define and quantify the kinetics of glucose and insulin, a physiological PK-PD model could be used. A physiologically accurate PK-PD model should include the main volumes of distribution, the main mechanisms of transport between compartments and the dominant irreversible clearance dynamics. In addition, physiologically-relevant PK-PD models should also capture the dynamic effects that in turn affect insulin sensitivity allowing researchers and

clinicians to understand and investigate the underlying factor to treat diabetes and other metabolic dysfunctions.

The physiology to be modelled includes:

- The effectiveness of insulin in maintaining glucose homeostasis (via insulin sensitivity metrics)
- Insulin secretion mechanism into the bloodstream from the pancreas
- Insulin clearance by the liver (first-pass hepatic extraction) and kidney
- Interstitial insulin kinetics which are defined by transcapillary transport into interstitial fluid, reaching tissue cells where it binds to activate glucose uptake
- Finally, insulin degradation at the peripheral cells.

The specific physiological effects to be investigated in this thesis:

- Parameters of insulin kinetics that influence model-based insulin sensitivity in overall metabolic PK-PD models
- The incretin effects that enhance insulin secretion
- Effects of insulin secretion and insulin clearance during haemodialysis (HD) treatment, which affects clearance.

Overall, it is essential to appreciate the physiology and interaction of glucose and insulin with other related mechanisms in investigating the underlying factors contributing to chronic diseases such as diabetes, cardiovascular disease and acute renal failure. For many years, clinicians and researchers have been working to develop and use model-based methods to increase understanding and aid decision support. The physiological foundation presented in this chapter is the basis of ensuring models are relevant.

Chapter 3. Model-Based Insulin Sensitivity Assessments

Insulin resistance (*IR*) has been widely accepted as the leading risk in the pathogenesis of Type 2 Diabetes Mellitus (T2DM) and cardiovascular disease (CVD) (DeFronzo and Ferrannini 1991). A long-term study has shown those who developed T2DM had an average initial *IR* 60% higher than those who remained healthy (Martin *et al.* 1992). An early diagnosis of this condition can reduce further complications through early intervention (ADA 2002; McAuley *et al.* 2002). Hence, simple and practical tests to identify insulin sensitivity would be clinically beneficial that enable researchers and clinicians to clearly understand and assess the underlying physiological and metabolic effects which caused these diseases. Such tests can be enabled by accurate, physiologically relevant metabolic models.

3.1 Introduction

Insulin sensitivity (S_I) is a measure of the efficiency of plasma insulin in reducing blood glucose concentration. There are many available tests measure this effect that all use somewhat different approaches (Bergman *et al.* 1979; Breda *et al.* 2002; Caumo *et al.* 2000; DeFronzo *et al.* 1979; Ferrannini and Mari 1998; Lotz *et al.* 2010; Matsuda and DeFronzo 1999; Matthews *et al.* 1985; McAuley *et al.* 2011; Pacini and Bergman 1986; Pacini and Mari 2003). Thus, it is necessary for the researcher/clinician to decide which test is best for any given condition in terms of intensity, cost, accuracy and physiological relevance. This decision thus implicitly states that there exists a hierarchy of such tests, each with different clinical method, and associated level of accuracy or resolution.

In addition, insulin sensitivity can be affected by lifestyle changes. Increases in exercise, healthier diet (Duncan *et al.* 2003; McAuley *et al.* 2002; Nishida *et al.* 2002; Tuomilehto *et al.* 2001) and weight loss (Camastra *et al.* 2005; Ferrannini *et al.* 2005; Ferrannini *et al.* 1997) are proven to improve sensitivity and thus reduce the prevalence or impact of type 2 diabetes. Hence, insulin sensitivity is affected by subject condition and a range of external variables.

S_I estimation has numerous uses in medical and clinical situations. However, high-resolution tests (i.e. EIC and hyperglycaemic clamp) that would be beneficial for clinical S_I measurement and monitoring are too intensive, long and costly for regular use. In addition, the sensitivity metrics yielded by the tests can be derived using models of the glucose-insulin pharmacodynamics that require more complex mathematical processes to evaluate than simpler clinical metrics. A comprehensive understanding and modelling of the variability in insulin sensitivity can assist clinical glycaemic control or intervention and minimise the associated risk of hyperglycaemia and/or hypoglycaemia, and thus mortality in critically ill patients. Thus, numerous clinically verified dynamic protocols have been developed to deliver data that enable S_I identification using various specific or generic glucose-insulin (PK-PD) models with mathematical methods and algorithms.

This chapter gives an overview on several dynamic model-based methodologies with different clinical application in assessing S_I . Such assessments are typically used mainly in research to assess the effects of treatment or medication on insulin sensitivity. Other tests are clinically focused, designed to screen populations and diagnose or detect the risk of developing diabetes. Thus, it is very important to observe sensitivity metrics (S_I) in various clinical and research settings.

3.2 Overview of current model-based S_I assessments

3.2.1 Euglycaemic clamp test (EIC)

The euglycaemic-hyperinsulinaemic clamp (EIC) was first proposed by DeFronzo *et al.* (1979). The test's concept based on constant infusion rate of insulin, while glucose has a variable infusion rate in order to “clamp” the plasma glucose concentration at a normal fasting plasma glucose concentration. The test is very flexible, as it can be performed at different target of glycaemic or insulinaemic concentrations to study changes in metabolic effects at these specific target states. It also can be combined with tracers or other drugs to assess the metabolic effect on glucose uptake. The test is considered as the gold standard method in assessing insulin sensitivity (Ferrannini and Mari 1998; Pacini and Mari 2003). In particular, the accuracy is good and it is highly repeatable (DeFronzo *et al.* 1979; Mari *et al.* 2001b; Monzillo and Hamdy 2003).

The EIC requires a cannula placed in the antecubital fossa to allow directly insulin and glucose infusions into bloodstream. A second cannula is placed in the dorsum of the hand for blood sampling. Heating of the hand surface is included in most study designs to maintain constant blood circulation, which reduces errors/noise in terms of measuring blood samples representative of overall, whole body concentrations. The surface of the hand is usually warmed using a purpose-built heated-hand-box, which heats and circulates air around the participant's hand. The temperature of the heated air is generally controlled between 40 and 70°C.

The test begins when insulin is infused at a rate proportional to the participant's size. The rate of insulin infusion is defined to achieve target of certain plasma insulin concentration (typically ~ 100 mU.L⁻¹), where healthy fasting concentrations are only relatively 5 to 10 mU.L⁻¹ and 50 to 70 mU.L⁻¹. This high concentration is supra-physiological and creates a hyperinsulinaemic that completely suppresses the hepatic glucose production and pancreatic insulin secretion. Thus, the glucose uptake rate is presumably equivalent the glucose infusion rate eliminating several variables. A glycaemic steady state needs to be achieved to use this assumption, which can take between 2-3 hours (Ader and Bergman 1987).

A glucose infusion begins 2-5 minutes after the insulin infusion to maintain euglycaemia between 4 and 5 mmol.L⁻¹. A closed loop system with glucose sampling every 10 minutes, and a continuous adjustment of the glucose infusion rate (DeFronzo *et al.* 1979; Ferrannini and Mari 1998) is required to reach and hold this euglycaemic steady state (Bergman *et al.* 1985). Measured insulin sensitivity signifies mainly peripheral sensitivity, as endogenous glucose production (EGP) is inhibited.

The EIC derived insulin sensitivity index (*ISI*) is defined as the mean glucose infusion at steady state, *M*, divided by the mean insulin concentration *I* at steady state:

$$ISI = \frac{M}{I} \quad 3.1$$

Based on above *ISI*, it is assumed that the infused glucose (*M*) is cleared by the average plasma insulin present over that hour. As a result, this ratio is physiologically relevant since EGP is

suppressed at very high insulin infusion rates while a steady state fasting glucose concentrations is well maintained. It thus minimises the variability due to EGP effect on the measured sensitivity index.

Overall, these tests are highly repeatable with a reported CV=6-10% (DeFronzo *et al.* 1979; Mari *et al.* 2001a; Monzillo and Hamdy 2003). It also offers a steady state assessment of a wide range of possible metabolic conditions as the glucose concentration can be clamped at any reasonable value, typically around 4.6 mmol.l^{-1} e.g. (McAuley *et al.* 2001). Thus, the EIC is used in evaluating other S_I tests' performance by correlated it to the clamp metric ISI .

3.2.2 Intravenous Glucose Tolerance Test (IVGTT)

Intravenous tests utilise an injection or infusion of glucose and/or insulin to cause a physiological perturbation of the metabolic system. Previous studies report that higher accuracy is generally achieved with intravenous (IV) tests compared to tests with oral dose stimulus due to the variability and difficulty in measuring the rate of glucose appearance after oral glucose ingestion (Breda *et al.* 2001; Caumo *et al.* 2000; Dalla Man *et al.* 2005b; Mari *et al.* 2001b). Thus, IV tests minimise modelling errors and variability in the resulting estimated S_I . However, they are invasive and time consuming to perform individually. Hence, they are typically used only in research settings, as they are too expensive and intense for a wider clinical use.

A simple model description of the glucose dynamics observed during an IVGTT protocol is illustrated in the minimal model (MM) presented by Bergman *et al.* (1979). Metabolic information on glucose kinetics and dynamics can be obtained by identifying the model parameters to match IVGTT data (Bergman *et al.* 1981; Pacini and Bergman 1986). The model differentiates between hepatic glucose balance (S_G^{MM}) and insulin-glucose mediated uptake, (S_I^{MM}). Insulin dependent glucose uptake is portrayed by a kinetic model of so-called 'insulin action'. The model captures a minimal description of physiological insulin secretion aspects. The MM was a very early approach used mathematical model for S_I measuring.

A typical IVGTT protocol includes cannula in the dorsum of the hand, and/or the antecubital fossa for blood samples and the application of an intravenous (IV) glucose input. A series of

blood samples are taken at 5-30 minutes resolution over 1.5 to 4 hours (depending on the protocol used) are taken to obtain the participant's basal condition (Bergman *et al.* 1981; Pacini and Bergman 1986). Then, a glucose bolus is given at $t=0$. The glucose bolus dose is typically dependent on the participant's weight or BSA (i.e. 0.3 g/kg body weight) to normalise this dose over a cohort. A further 4-50 samples are taken after the glucose bolus. Glucose and insulin samples were assayed to quantify S_I using a variety of methods. S_I is usually estimated using the non-linear least-squares parameter identification method with the MM proposed by Bergman *et al.* (1979). The Minimal Model (MM) is defined:

$$\dot{G} = -S_G^{MM}(G - G_B) - XG + \frac{P_X}{V_G} \quad 3.2$$

$$\dot{X} = -p_2X + p_3(I - I_b) \quad 3.3$$

where Table 3.1 defines the variables.

Insulin sensitivity is estimated as a function of identified values for p_2 and p_3 :

$$S_I^{MM} = \frac{p_3}{p_2} \quad 3.4$$

Table 3.1 Nomenclature of Equations 3.2-3.4.

| Parameter | Description | Unit |
|----------------|---|---------------------------------------|
| G | Plasma glucose concentration | mmol.L ⁻¹ |
| G_b | Basal plasma glucose concentration | mmol.L ⁻¹ |
| X | 'Action' of insulin i.e. insulin effect on net glucose decay | mU.L ⁻¹ |
| P_x | Rate of intravenous glucose input | mmol.min ⁻¹ |
| S_G^{MINMOD} | Decay of glucose concentration at basal insulin | min ⁻¹ |
| p_2 | Total rate of insulin transport into the in-accessible compartment (interstitial) and efficiency of insulin into a single compartment | min ⁻¹ |
| p_3 | Total clearance of insulin from the in-accessible compartment | L.mU ⁻¹ .min ⁻² |
| V_G | Volume of glucose distribution | L |
| $I-I_b$ | Plasma insulin concentration above basal | mU.L ⁻¹ |

There are several issues noted since the method was first validated (Bergman *et al.* 1987; Docherty *et al.* 2011; Donner *et al.* 1985; Finegood *et al.* 1984; Saad *et al.* 1994). The most significant issues are the over-parameterisation of the MM that causes the inability to segregate between insulin and non-insulin mediated glucose disposal and the resulting convergence to local, not global, error minima during the non-linear least-square identification process (Cobelli *et al.* 1998; Mari 1997; Pillonetto *et al.* 2002; Quon *et al.* 1994). The issue of over-parameterisation can be somewhat mitigated by the application of complex Bayesian identification methods (Cobelli *et al.* 1999; Erichsen *et al.* 2004; Pillonetto *et al.* 2002). However, no complete solutions are available to remedy these issues that also significantly improve the diagnostic resolution and repeatability of these tests in clinical and research settings. Hence, MM methods are limited their ability to accurately capture S_I .

Both the S_I^{MM} and S_G^{MM} metrics are coefficients of functions of glucose concentration and quantify glucose disappearance over time. Thus, parameter value trade-offs can occur during the parameter identification process. As a result, intra-participant repeatability between tests is poor (Gelding *et al.* 1994; Monzillo and Hamdy 2003; Pillonetto *et al.* 2003; Quon *et al.* 1994). The net error increases further due to the discrepancy of clinical data to measure noise from assay error suspected from physiological mixing, ineffective cannula flushing or decay prior to assay.

The IVGTT itself is ideal physiologically, as it includes the highly dynamic endogenous insulin secretion impulse response of the glucose-insulin PD system to a glucose bolus. When a glucose bolus is injected intravenously it triggers the bi-phasic endogenous insulin response (Ferrannini and Mari 2004). Thus, IVGTT delivers data from which both phases of β -cell function can be distinctively identified. Therefore, it also provides an opportunity to measure and assess pancreatic performance. However, further modifications to improve test performance need to be considered so that the MM is more physiologically relevant to the research studies (Callegari *et al.* 2003; Caumo and Cobelli 1993; Caumo *et al.* 1999; Ferrannini *et al.* 1988; Mari 1998), as the test becomes impractical for application in wider clinical settings (ADA 1998).

Overall, as a result, several studies have shown an ambiguous correlation between S_I^{MM} and the gold standard EIC ($R=0.44-0.92$) (Bergman *et al.* 1987; Finegood *et al.* 1984; Foley *et al.* 1985;

Galvin *et al.* 1992; Katz *et al.* 2000) with more results in the lower range. The outcome is dependent on the subgroups and cohorts studied, and potentially the specific protocols used. This wide range of correlation implies some question on the tests accuracy and/or robustness. The over-parameterisation issue using the MM and the limited accessibility to complex computer parameter identification algorithms have constrained the acceptance of the IVGTT for uses outside of clinical trials and research. Thus, caution must be practised in interpreting S_I^{MM} and S_G^{MM} values.

3.2.3 Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test (OGTT) is clinically practical, easier for individual use and research relevant in investigating the incretin effects. Currently, various doses and sampling protocols are used either in research or clinical settings. However, a standard OGTT consists of ingestion of a 75-g or 50-g glucose drink, followed by blood samples (usually 0, 30, 60, 120, 150, 180 minutes) for 1-3 hours (Pacini and Mari 2003). Blood glucose and plasma insulin concentrations, and sometimes C-peptide concentrations, are sampled. Due to its relative ease of administration, it is the current method of choice for a clinical diagnosis of diabetes as recommended by the American Diabetes Association (ADA 2006a). However, these assessments do not strictly measure a physiological effect, but only reflect the outcome of these effects. In turn, some studies have proposed mathematical models to measure and investigate the physiological effects using an OGTT data to directly assess S_I .

A series of model modifications have been suggested to apply the MM to OGTT responses. Thus, an initial study by Caumo *et al.* (2000) modified the MM to model glucose appearance in bloodstream have also been developed. Follow by other model-based methods for parameter estimation from OGTT data (Breda *et al.* 2001; Dalla Man *et al.* 2005a; Mari *et al.* 2005; Mari *et al.* 2001b). In practice, the dynamic and variable rate of glucose absorption from gut to the bloodstream is practically immeasurable and causes variability in results and computational parameter identifiability issues. In particular, the identified S_I based on these proposed models is directly affected by this variable rate of glucose absorption. Thus, variations in absorption or appearance rates play a major confounding role in modelling the response to oral glucose-insulin stimulus.

Figure 3.1 illustrates schematic diagram of the minimal model used to interpret the insulin-modified, Frequently Sampled Intravenous Glucose Tolerance Test, (FS-IVGTT), (Cobelli *et al.* 1984; Saad *et al.* 1994) and the Meal Glucose Tolerance Test, (MGTT), or OGTT (Breda *et al.* 2001; Caumo *et al.* 2000). The rate of glucose input to the model is from the impulsive dose of glucose (P_X) for the insulin-modified FS-IVGTT. The MGTT/OGTT the rate of appearance of absorbed glucose (R_{ABS}) determines (P_X) to the model. Other kinetic transport parameters are shown k_{1-5} .

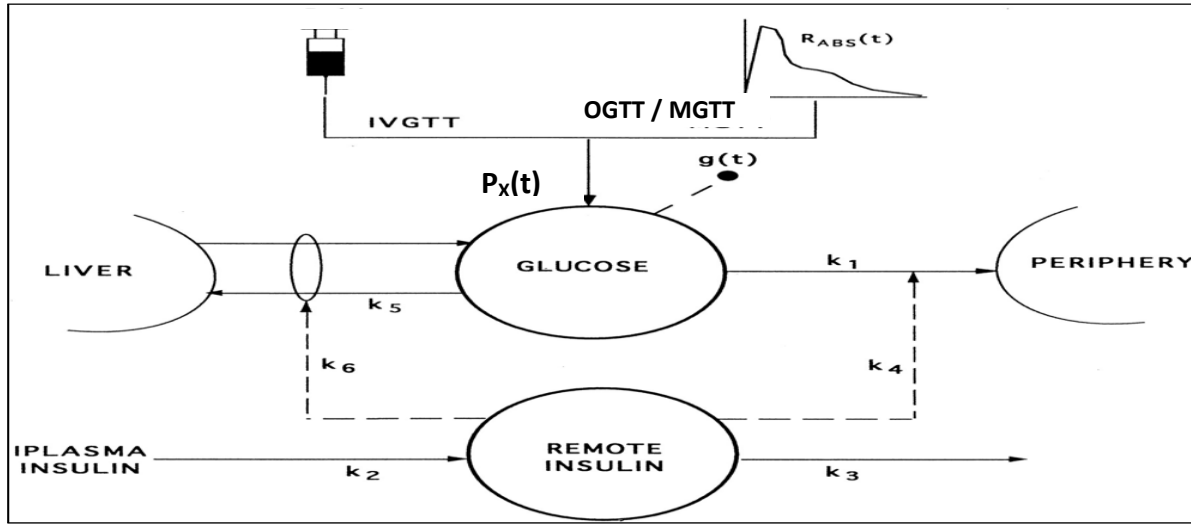


Figure 3.1 Schematic diagram of the minimal model used to interpret the insulin-modified FS-IVGTT (Saad *et al.*, 1994) and the MGTT/OGTT by Caumo *et al.* (2000).

The challenge in using the MM in Equation 3.5 to analyse oral MGTT/OGTT data is to mathematically describe the rate of appearance of absorbed glucose reaches the systemic circulation as (P_X) (as in Figure 3.1). Thus, Caumo *et al.* (2000) suggested a modification on glucose input rate of MM to anticipate the rate of oral glucose appearance in bloodstream in order to assess S_I .

The resulting mathematical expression in assessing OGTT derived S_I is defined Caumo *et al.* 2000:

$$S_{I(oral)}^{MM} = \frac{f \cdot D_{oral} \frac{AUC[\Delta g/g]}{AUC[\Delta g]} - GE \cdot AUC[\Delta g/g]}{AUC[\Delta i]} \quad 3.5$$

where AUC denotes the area under the curve calculated from time zero to the end of the test. $GE=p_I V$ is the glucose effectiveness ($\text{mL.kg}^{-1}.\text{min}^{-1}$). D_{oral} is the dose of ingested glucose per unit of body weight (mL.kg^{-1}), and f is the fraction of ingested glucose that actually appears in the systemic circulation (i.e. survives gastrointestinal absorption and first-pass hepatic uptake). As individual estimates of GE and f are not available, these values are assumed from prior studies. $GE=0.024$ ($\text{dL.kg}^{-1}.\text{min}^{-1}$) (Best *et al.* 1996), and $f=0.8$ (Kolterman *et al.* 2003).

This approach allows a direct measurement of IR during a more physiological state, and has been intensively used in considerable research and validation studies. Validation using a meal input against the insulin-modified FS-IVGTT was performed in healthy subjects with good correlations of $R=0.89$ (Caumo *et al.* 2000) and $R=0.75$ (Dalla Man *et al.* 2002). The OGTT version was validated against EIC, resulting in a correlation of $R=0.81$ (Dalla Man *et al.* 2005b) and yielded a moderate level of repeatability with $CV=12-15\%$ in a study by Breda *et al.* (2001).

3.2.4 Dynamic Insulin Sensitivity and Secretion Test (DISST)

The simple and low-cost test DISST is applicable in clinical and research settings and measures S_I and insulin secretion. The DISST protocol uses a series of blood samples to measure the participants' response to administered glucose and insulin boluses. A simple version of DISST that measured 5 blood samples taken over a 35-minutes protocol uses low-dose, intravenous glucose (10-g) and insulin (1U) boluses as inputs. Thus, it is comparatively short and for less intense than the gold standard EIC protocol.

The DISST model and identification method allow the intermittent sampling by fitting and refining physiological responses to the measured data (Lotz 2007; Lotz *et al.* 2010). The PK-PD of DISST model accounts for participant-specific losses of insulin to the hepatic and renal, saturation of insulin clearance at high concentrations, and diffusion and mass conservation of insulin between the plasma and the interstitial (Lotz *et al.* 2010). It is also possible to assess β -cell function using established method from Van Cauter *et al.* (1992), using C-peptide assays. Thus, it provides S_I and endogenous secretion, which no other test listed can do.

Participants had a cannula inserted into the antecubital fossa for blood sampling and bolus infusion. Blood samples were collected at $t=0, 10, 15, 25$, and 35 minutes as glucose, insulin and C-peptide concentrations were measured using these samples. A 10-g bolus of IV glucose was given at $t=5$ minutes and a 1 U Actrapid insulin bolus was given immediately after $t=15$ -minute sample. After the test, all participants were required to remain at the clinic for 30 minutes and a small meal or snack was provided to them.

The parameter identification methods of dynamic tests, such as the DISST, are sensitive to the timing of samples. Thus, the actual samples times are recorded for reference. The iterative integral method (IIM) or integral method is used to identify model-based S_I , glucose distribution volume (V_g) and first-pass hepatic insulin extraction (x_L) and subsequent hepatic insulin clearance (n_L). Metrics of β -cell function are derived from insulin secretion profiles that are deconvoluted from interpolated C-peptide measurements using the established method of Van Cauter *et al.* (1992).

The DISST model defines the PK-PD of C-peptide, insulin, and glucose. The model relates the rate of glucose decay to the concentration of insulin available in the interstitium to provide a metric of insulin sensitivity. The model equations are defined:

$$\dot{C} = -(k_1 + k_3)C + k_2Y + \frac{U_{en}}{V_p} \quad 3.6$$

$$\dot{Y} = k_1C - k_2Y \quad 3.7$$

$$\dot{I} = \frac{n_I}{V_p}(Q - I) - n_KI - n_L \frac{I}{1 + \alpha_I I} + \frac{U_{ex}}{V_p} + (1 - x_L) \frac{U_{en}}{V_p} \quad 3.8$$

$$\dot{Q} = \frac{n_I}{V_q}I - (\frac{n_I}{V_q} + n_c)Q \quad 3.9$$

$$\dot{G} = p_{gu}(G_b - G) - S_I(GQ - G_bQ_b) + \frac{P}{V_g} \quad 3.10$$

where all variables and parameters are defined in Table 3.2.

Table 3.2 Nomenclature of the DISST model in Equations 3.6-3.10

| Parameter | Description | Unit |
|-----------------|--|----------------------|
| C | Plasma C-peptide concentration | mU.L^{-1} |
| Y | Interstitial plasma C-peptide concentration | mU.L^{-1} |
| I | Plasma insulin concentration | mU.L^{-1} |
| Q | Interstitial plasma insulin concentration | mU.L^{-1} |
| G | Glucose concentration | mmol.L^{-1} |
| k_1, k_2, k_3 | kinetic parameters | min^{-1} |
| U_{en} | Rate of insulin secretion | mU.min^{-1} |
| V_p | Distribution volume of insulin in the plasma | L^{-1} |
| n_I | Transition rate of insulin between plasma and interstitial | L.min^{-1} |
| n_K | Rate of renal insulin clearance | min^{-1} |
| n_L | Rate of hepatic insulin clearance | min^{-1} |
| α_I | Saturation of hepatic insulin clearance | L.mU^{-1} |
| n_C | Rate of insulin clearance to cells | min^{-1} |
| U_{ex} | Bolus input of insulin | mU |
| x_L | Hepatic first-pass extraction of insulin | 1 |
| p_{gu} | Glucose-dependent rate of glucose disposal | min^{-1} |
| P | Glucose bolus | mmol |
| V_g | Distribution volume of glucose | L |
| G_b | Basal glucose concentration | mmol.L^{-1} |
| Q_b | Basal interstitial insulin concentration | mU.L^{-1} |

First, the kinetic parameters of Equations 3.6-3.7 are quantified using functions of participant weight, height, gender, and age that were defined by Van Cauter *et al.* (1992). Then, a piecewise linear interpolation of the C-peptide data was used with these kinetic values in a deconvolution calculation to produce an endogenous insulin secretion profile. Finally, S_I , V_g , n_L , and x_L (from Equations 3.8-3.10) were identified using the deconvoluted U_{en} profile, insulin and glucose measured data, and the iterative integral method (Hann *et al.* 2005; Lotz *et al.* 2010). The overall S_I identification process from PK-PD of the DISST model is illustrated in Figure 3.2.

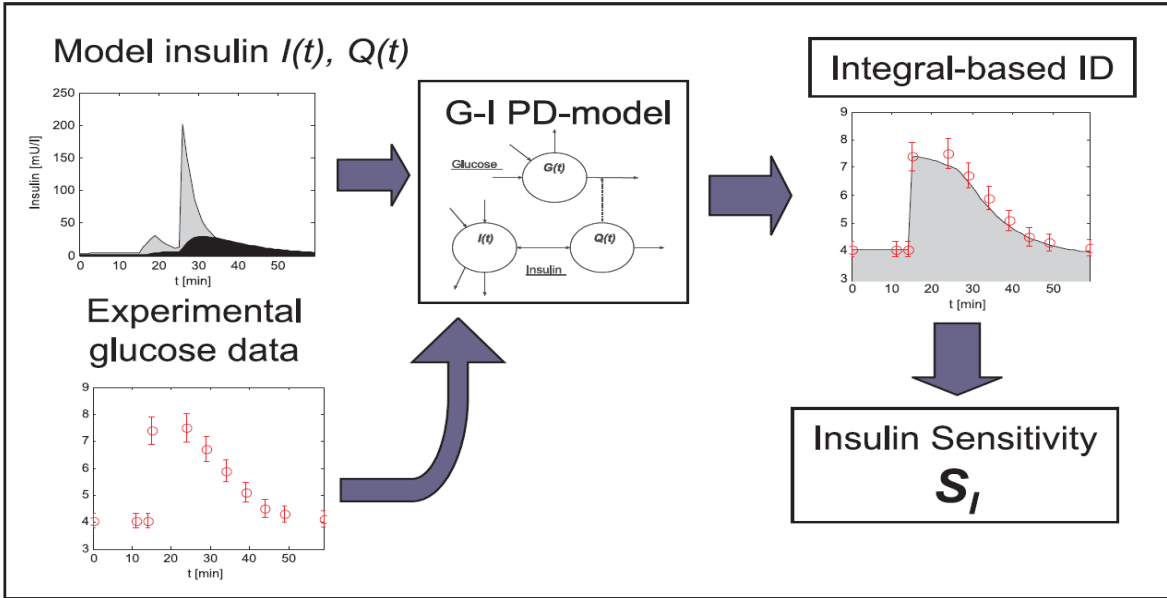


Figure 3.2 The overall method of estimating the model-based S_I . Modeled peripheral insulin Q , and sampled glucose concentration data are combined in the glucose/insulin pharmacodynamic model. S_I is estimated by fitting the model with the iterative integral method (IIM).

A strong correlation between S_I identified by the DISST and the glucose clamp as reported ($R=0.82$) by McAuley *et al.* (2011) with CV ranges of ~8-11% (Lotz 2007). Table 3.3 shows the DISST performances in comparison to the well-known HOMA metric for insulin sensitivity with correlation to the EIC. The HOMA result reported within the ranges in the literature (Bonora *et al.* 2000; Katsuki *et al.* 2001; Lotz *et al.* 2008; Mari *et al.* 2001b; Matthews *et al.* 1985; Pacini and Mari 2003).

Table 3.3 Pearson correlations and c-ROC (area under the curve) values between the sensitivity metrics derived during the DISST study.

| | EIC R (c-ROC) |
|--------------|---------------------------------------|
| DISST | 0.82 (0.96) |
| HOMA | 0.60 (0.92) |

The DISST model and methodology is simple, requires minimal data to assess S_I and is thus practically useful in both clinical and research settings. The test yields more information regarding glucose and insulin responses to stimuli than other available tests. It also compares well against other established and more intensive physiological approaches (Ferrannini and Mari

1998; Pacini and Mari 2003; Wallace and Matthews 2002). If the DISST were to be applied widely, it could potentially enhance over understanding of the pathophysiology of type 2 diabetes and characterise subgroups among the heterogeneous cohort of *IR* individuals.

3.2.5 Homeostasis Model Assessment (HOMA)

HOMA is a computer-solved model that estimates insulin secretion and insulin sensitivity based on the interactions between fasting plasma glucose and insulin. The test index was derived from a physiological whole body glucose-insulin model, developed based on physiological data (Matthews *et al.* 1985). The same physiological model was also used in the assessment of CIGMA. Other test approaches measure S_I in the presence of stimuli. However, HOMA only gives an estimate of basal insulin resistance. Caution should be used when making comparisons between studies due to variations in infusion protocols, sampling procedures and assay types used in different studies.

The HOMA index is obtained from a simple equation of the fasting plasma glucose concentration (G_b) (mmol.L⁻¹) and the fasting plasma insulin concentration (I_b) (mU.L⁻¹), is defined:

$$HOMA - S_I = \frac{22.5}{G_b I_b} \quad 3.11$$

It is reported that the initial process in identifying *IR* index (or HOMA- S_I) requires a series of samples measured on consecutive days (Wallace *et al.* 2004), which increases the clinical intensity. Due to its simplicity and comparability across studies, the HOMA measure is used in many research studies, as well as clinically.

In addition, a separate HOMA index of β -cell function, HOMA-%B, can be estimated similar to Equation 3.12:

$$HOMA - \%B = \frac{20I_b}{G_b - 3.5} \quad 3.12$$

HOMA has been validated against the EIC. A wide spread of correlations values were obtained between a very poor $R=0.22$ and a very good $R=0.93$ (Bonora *et al.* 2000; Mari *et al.* 2001b; Mather *et al.* 2001; Matthews *et al.* 1985; Pacini and Mari 2003). In addition, average to poor CV values has also been reported between 10-20% (Bonora *et al.* 2000; Lotz *et al.* 2008; Matthews *et al.* 1985; Wallace *et al.* 2004).

Currently, HOMA is used by clinical researchers, primarily to quickly and cheaply estimate IR . A relatively high CV and the lack of real-time measurements limit the tests diagnostic use. The HOMA metric assumes that S_I will be inversely proportional to both insulin and glucose concentrations. However, the lack of test stimulus means that the effect of the pulsatile release of insulin (Del Prato *et al.* 2002; Meier *et al.* 2005) and assay error are significant and adverse confounding effects. In addition, the resolution of the HOMA metric is poorest in the region of maximum clinical utility. Thus, in many cases a simple fasting glucose is used instead, as it is a simple test and practical in a general clinical setting.

3.2.6 Continuous Infusion Glucose Model Assessment (CIGMA)

CIGMA is an intravenous test that consists of a relatively low dose infusion of glucose over 60 minutes ($5 \text{ mg.kg}^{-1}.\text{min}^{-1}$) to mimic a postprandial state. Sample of glucose and insulin were measured during the final 15 minutes which is approximately steady state (Hosker *et al.* 1985). Then, the test data are compared to known physiological data using a model of whole body glucose homeostasis (similar to Equation 3.11) that accounts for the glucose infusion rate and clearance. CIGMA has a reported repeatability of CV=17-21% (Hosker *et al.* 1985; Nijpels *et al.* 1994) and reported correlations to the EIC vary between $R=0.66-0.87$ (Hosker *et al.* 1985; Nijpels *et al.* 1994).

The estimated S_I obtained is likely a good match to the true combined peripheral and hepatic sensitivity due to the physiological glycaemic state attained during the test. The test also is restricted and not applicable in individuals with a weak pancreatic insulin response or type 1 diabetes (Ferrannini and Mari 1998). Thus, these limitations make it less useful and unreliable in clinical settings, although the relative simplicity, safety and potential physiological relevance make it appealing in research studies and some limited clinical situations.

3.3 Physiologic role of S_I in model-based assessments and other uses

Insulin sensitivity is not a discrete metric that can be assessed with a simple, well defined test. It is a concept to quantify the body's ability to reduce blood glucose concentrations in response to insulin secretion. This definition is very broad and includes many underlying physiological effects that contribute to the whole body response. Three major effects contribute to the overall whole body sensitivity and response to glucose, EGP, insulin sensitivity and β -cell function. Highly complex tests, such as EIC, IVGTT, and Oral Minimal Model assessment cannot differentiate between these three physiological effects without tracer labelled glucose and C-peptide sampling. The DISST can segregate endogenous insulin secretion, which the others cannot. In turn, it is very difficult to compare such varied methods in assessing S_I , as they also do not always measure the same physiologic effects.

Insulin sensitivity in a fasting state can be significantly different (steady-state) than in the perturbed state (dynamic state) (Ferrannini and Mari 1998). In addition, S_I can be different for a more physiological oral perturbation as oral glucose triggers insulin-stimulated gastrointestinal hormones (i.e. incretins) (McIntyre *et al.* 1965; Muscelli *et al.* 2008; Nauck *et al.* 1986; Vilsbøll and Holst 2004) that do not occur with IV glucose (Breda *et al.* 2001). It is essential to include all relevant physiological effects in the model assessment to portray the kinetics of glucose-insulin and identify S_I . Thus, S_I can be evaluated in many different ways depending on clinical or/and research applications.

One of the clinical roles of model-based S_I is to capture the glucose-insulin metabolic system dynamics that accurately diagnose, monitor and predict blood glucose concentrations in glycaemic control of critically ill patients. Thus, a common aspect that portrays model success in modelling glucose/insulin pharmacodynamic-pharmacokinetic (PD-PK) is the model's ability to capture the glycaemic response to insulin, which is often expressed in terms of overall, whole-body insulin sensitivity (Chase *et al.* 2008; Evans *et al.* 2011; Fisk *et al.* 2012; Hovorka *et al.* 2007; Le Compte *et al.* 2009; Plank *et al.* 2006).

It is suggested that IV tests have the highest repeatability, as the test protocols controls many variables. The highest repeatability reported is for the EIC due to the suppression of all

endogenous glucose and insulin secretion (reducing all the unknown dynamics). However, Cobelli *et al.* (1998) and Mari (1997) found that IVGTT triggers unmodeled regulatory responses that negatively affect model fitting. Thus, the potential remedy of these problems to improve the model accuracy is to modify the protocol, rather than creating complex modelling or clinical settings (Lotz *et al.* 2006).

Oral-based S_I assessments are more variable due to the variable appearance rate of glucose in bloodstream. The rate of gastric absorption is difficult to measure without tracers and can be very variable in different individuals. Thus, any estimation of this rate involves simplified assumptions contributes to a high variability in the overall test result. However, a larger number and heterogeneous of samples used in determining the S_I metric can somewhat reduce variability by fully capturing the dynamic response without losing its functionality in screening T2D/IGT.

3.4 Summary

Overall, different studies have used different methods for assessing S_I . However, it has been suggested (Groop *et al.* 1993) that the ideal method for measuring S_I should satisfy five criteria:

1. High insulin concentrations to stimulate glucose metabolism
2. Observe the distinction between peripheral and hepatic insulin sensitivity
3. Measure steady-state conditions
4. Capture the physiologic glucose equilibrium system after IV/oral ingestion
5. Reach a hyperglycaemic state which is not overtly non-physiological

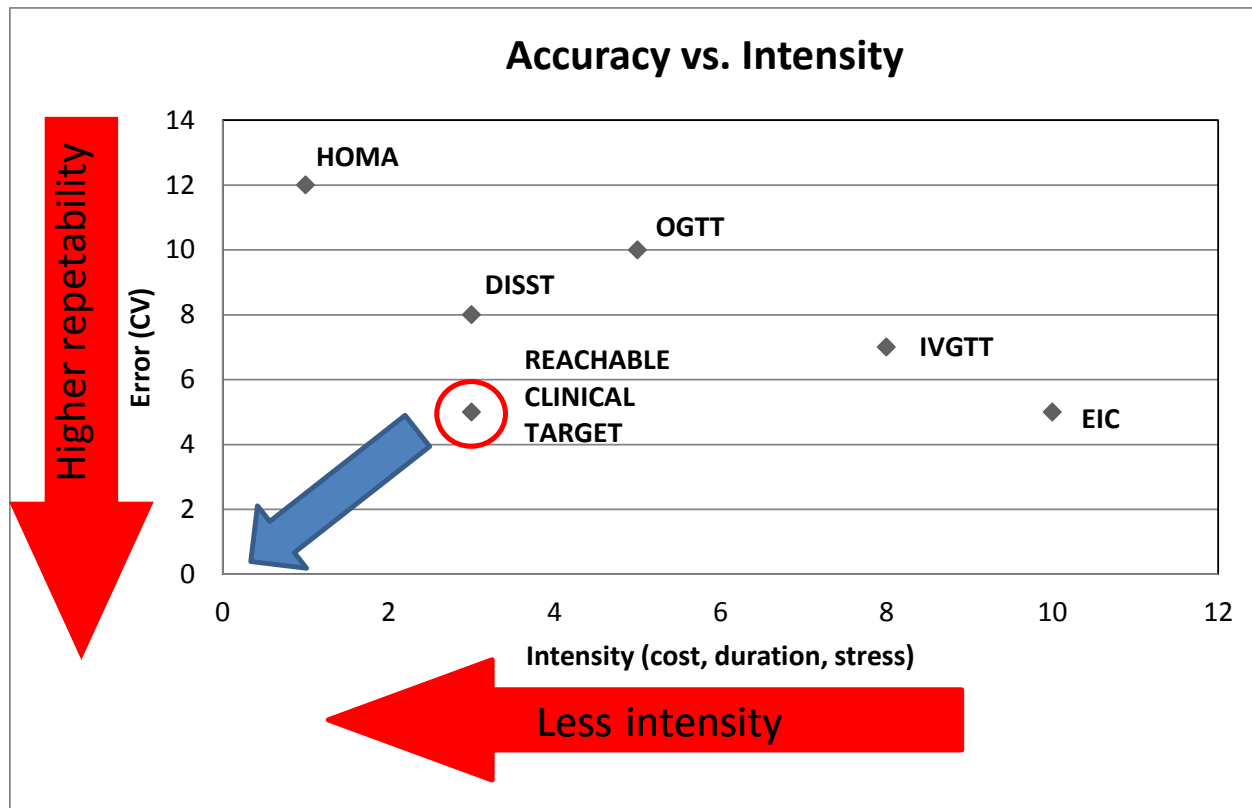


Figure 3.3 Comparison of different S_I assessments and target area for a better clinical test. The points are plotted according to the test's intensity and accuracy in terms of repeatability (CV in %). Intensity is a composite symbolic measure of cost, duration, and overall stress (labour) involved.

Several different methods to measure S_I have been proposed to date with varied complexity and accuracy as in Figure 3.3. Tests that quantify S_I directly use intravenous or oral glucose administration and glucose, insulin and C-peptide measurements in order to capture the dynamic metabolic response to the perturbation. It can be concluded that the more repeatable tests such as EIC or IVGTT are only used in research settings, as they are too complex, clinically intense and costly for regular or widespread clinical use.

A clinically useful test that is repeatable and provides a good resolution to monitor small changes in S_I would assist an early and more accurate diagnosis of *IR*. Such a test could be engineered by considering the good aspects of available accurate research tests, while reducing their intensity and also minimising errors identified in their methods.

Chapter 4. Interstitial Insulin Action

Delayed glucose uptake correlates closely with the changes in interstitial insulin rather than with plasma insulin, showing how interstitial insulin levels determine the uptake rate of glucose in the periphery (Castillo *et al.* 1994; Yang *et al.* 1994). From a model-based perspective, the critical parameters values that affect glucose uptake are transcapillary diffusion rate (n_I) and insulin binding saturation (α_G). These two parameters thus influence the identified patient-specific insulin sensitivity (S_I). This chapter uses glucose-insulin and C-peptide PK-PD models to analyse these parameters to determine a best set of parameter values.

4.1 Introduction

The DISST is a dynamic insulin sensitivity test using a low dose of insulin bolus with the addition of a low dose glucose bolus (Lotz 2007). Measurements are taken for blood glucose, plasma insulin and C-peptide. Insulin sensitivity, S_I , and other patient-specific parameters are then calculated from a physiological model of C-peptide-insulin-glucose kinetics and dynamics. In a Monte Carlo study, the S_I value from the DISST model achieves a correlation of $R=0.98$ (90%CI: [0.97, 0.98]) in S_I to the ISI of the gold-standard euglycaemic clamp (EIC) when fit to clamp data (Lotz *et al.* 2008). In the DISST itself, the mean intra-patient variability between S_I in different DISSTs is reported to be 11% compared to a value of 6% for the EIC (Lotz *et al.* 2010). Hence, the DISST and its identified S_I metric accurately capture metabolic response.

As measurements are only available for blood glucose, plasma insulin and C-peptide, many model parameters must be determined *a-priori*. This requirement limits the number of patient-specific parameters to be identified, and thus improves the model identifiability (Docherty *et al.* 2011). The kinetics of insulin and C-peptide have been extensively studied and are generally well understood (Duckworth *et al.* 1988; Duckworth and Kitabchi 1981; Van Cauter *et al.* 1992). However, interstitial insulin and pharmacokinetics of insulin receptors binding still present many unknowns (Duckworth *et al.* 1998). In particular, dysfunctions at the cellular level are largely speculated to contribute to insulin resistance (Barrett *et al.* 2009; Black *et al.* 1982; Brownlee and Hirsch 2006; Bryant *et al.* 2002; Duckworth *et al.* 1998).

Saturation in insulin-mediated glucose removal has been observed at varying levels (Docherty *et al.* 2010; Natali *et al.* 2000; Nestler *et al.* 1988; Prigeon *et al.* 1996; Rizza *et al.* 1981; Transberg *et al.* 1981). However, this effect is not taken into account by the EIC. Therefore, the EIC can underestimate insulin sensitivity as levels of plasma insulin are induced beyond physiological levels and beyond any linear relationship between glucose disposal and plasma insulin level (Docherty *et al.* 2011; Prigeon *et al.* 1996; Rizza *et al.* 1981). The model developed for the DISST also ignores insulin effect saturation, although the low insulin doses used were intended to avoid reaching saturation levels during DISST pilot study thus negotiating its need in that study (Lotz 2007) later uses of that model at higher doses have included insulin saturation (Lin *et al.* 2011).

This chapter investigates interstitial insulin action and its influence on model-based insulin sensitivity. The analysis uses the data from the DISST clinical pilot study (Lotz 2007) and the Intensive Control Insulin-Nutrition-Glucose (ICING) model (Lin *et al.* 2011) and, from these inputs, develop a modeled interstitial insulin dose-response that best links insulin action in plasma to response in blood glucose levels. The critical parameters influencing interstitial insulin pharmacokinetics (PKs) are saturation in insulin receptor binding and the plasma-interstitium diffusion rate and population values for these parameters are found from this analysis.

4.2 Subjects and Methods

4.2.1 Participants

Data from 17 participants were used in this analysis. The participants were originally recruited for the pilot study of DISST (Lotz *et al.* 2010). Each participant underwent at least two DISST tests on separate days. All tests were performed between 9-11am after an overnight fast from 10pm. The participant's weight, height and blood pressure were taken.

Three different dosing frequencies were used in the pilot study. A low dose test involves an intravenous glucose injection of 5g followed by an intravenous insulin injection of 0.5U. A median dose test uses 10g of glucose and 1U insulin. A high dose test uses 20g glucose and 2U insulin. Table 4.1 summarizes the tests these patients underwent. More details on the patient cohort and the pilot study can be found in Lotz (2007) and Lotz *et al.* (2010).

This study was approved by the Upper South Regional Ethics Committee, New Zealand.

Table 4.1 Participant details summary from the pilot DISST study.

| Group | Number (M/F) | Age median,[IQR] (years) | BMI median, [IQR] (kg.m ⁻²) | Test Dose | | |
|-----------|-----------------|--------------------------------|---|-----------|--------|------|
| | | | | low | medium | high |
| NGT* | 13 (4/9) | 41, [29, 51] | 25, [23, 27] | 7 | 24 | 5 |
| T2DM/IFG* | 4 (1/3) | 56, [52, 58] | 32, [28, 35] | 4 | 4 | 2 |

*NGT = normal glucose tolerance. T2DM = Type 2 diabetes mellitus. IFG = impaired fasting glucose.

4.2.2 Test procedure

Measurements in blood glucose (G), plasma insulin (I) and plasma C-peptide (C) were taken during the tests. Initially, glucose was administered as a 50% solution. Insulin was dosed in two steps to minimise dilution errors. First, 0.5ml (50U) of insulin (Actrapid, NovoNordisk) was drawn in a 1ml syringe and diluted with 49.5 ml saline to obtain a 1U/ml solution. Second, the test dose was drawn (0.5ml, 1ml or 2ml) in a 3ml syringe.

A cannula was inserted into the antecubital fossa. Two baseline blood sample (8 ml) were taken at $t=-10$ and $t=0$ minutes. Glucose administered at $t=0$ min and insulin $t=10$ min just after baseline samples taken at those times. Apart from baseline samples, blood was sampled during the test at $t=5, 10, 15, 20, 25, 30, 35$ and 45 minutes. Sampling and administration was performed on the same cannula. It was flushed first with the participant's own blood, then with saline after each bolus input to avoid any contamination. In particular, this approach minimises errors due to insulin and glucose binding to inner walls of tubes (NovoNordisk 2002). Each participant was given a snack after the test.

Blood samples were sent to the laboratory immediately or frozen at -80°C . In the laboratory, these blood samples were centrifuged and plasma serum was separated for plasma insulin and plasma C-peptide. Glucose was analysed by an enzymatic glucose hexokinase assay (Abbott). Plasma insulin and plasma C-peptide were analysed with an enhanced chemiluminescence immunoassay (ECLIA) (Roche 2004).

4.2.3 Participant specific parameter identification

Participant specific parameter identification is performed in three stages using measured G (mmol.L⁻¹), I (mU.L⁻¹) and C (pmol.L⁻¹) data.

In the first stage, endogenous insulin secretion (U_{en}) is calculated using the C-peptide model in Equations 4.1–4.2 adapted from Docherty *et al.* (2011).

$$\dot{C} = -(k_1 + k_3)C + k_2Y + U_{en} \quad 4.1$$

$$\dot{Y} = k_1C - k_2Y \quad 4.2$$

In the second stage, participant specific first pass hepatic clearance (x_L) and liver insulin clearance (n_L) are fitted to plasma insulin measurements using insulin and glucose infusions, U_{ex} and P . U_{en} is calculated in the first stage by deconvolution. The pharmacokinetic (ICING) models defined in Equations 4.4–4.5 are used in this stage, and a good fit will have modeled I in good agreement with plasma insulin measurements. An integral based parameter identification method (Hann *et al.* 2005) is used for commencement identification of x_L and n_L .

In the third stage, patient-specific insulin sensitivity (S_I) is identified by fitting the pharmacodynamic (ICING) model defined in Equation 4.3 to blood glucose measurements using identified values from Equations 4.4–4.5 to estimate $Q(t)$ and other known inputs in Equation 4.3. The same integral fitting method is used for the identification. Finally, it is important to note that Equation 4.3 also includes insulin effect saturation in the term α_G . The ICING model is thus defined:

$$\dot{G} = -p_G G - S_I G \frac{Q}{1 + \alpha_G Q} + \frac{P + EGP - CNS}{V_G} \quad 4.3$$

$$\dot{Q} = n_I(I - Q) - n_C \frac{Q}{1 + \alpha_G Q} \quad 4.4$$

$$\dot{I} = -n_K I - \frac{n_L I}{1 + \alpha_I I} - n_I(I - Q) + \frac{U_{ex}}{V_I} + (1 - x_L) \frac{U_{en}}{V_I} \quad 4.5$$

All parameters are *a-priori* values are defined in Table 4.2. Table 4.3 defines the exogenous input for the tests.

Table 4.2 Parameter values and descriptions for the pharmacodynamics/kinetics (ICING) models and C-peptide model.

| Parameter | Value | Unit | Description |
|-----------------|-----------------|------------------------------------|---|
| G | stimulated | mmol.L^{-1} | Blood glucose concentration |
| Q | stimulated | mU.L^{-1} | Interstitial insulin concentration |
| I | stimulated | mU.L^{-1} | Plasma insulin concentration |
| C | stimulated | pmol.L^{-1} | Plasma C-peptide concentration |
| Y | stimulated | pmol.L^{-1} | Interstitial plasma C-peptide concentration |
| U_{en} | stimulated | mU.min^{-1} | Endogenous insulin secretion rate |
| p_G | 0.006 | min^{-1} | Non-insulin mediated glucose removal |
| EGP | 1.16 | mmol.min^{-1} | Endogenous glucose production rate |
| CNS | 0.3 | mmol.min^{-1} | Central nervous system glucose uptake |
| V_G | 13.3 | L | Plasma glucose distribution volume |
| V_I | 3.15 | L | Plasma and interstitial insulin distribution volume |
| α_I | 0.0017 | L.mU^{-1} | Hepatic insulin clearance saturation parameter |
| n_K | 0.0542 | min^{-1} | Renal insulin clearance rate |
| n_L | 0.1578 | min^{-1} | Hepatic insulin clearance rate |
| x_L | 0.67 | | Fractional first-pass hepatic insulin extraction |
| n_I | identified | min^{-1} | Plasma-interstitial diffusion rate |
| n_C | identified | min^{-1} | Interstitial insulin degradation rate |
| α_G | identified | L.mU^{-1} | Insulin binding saturation parameter |
| S_I | identified | $\text{L.mU}^{-1}.\text{min}^{-1}$ | Insulin sensitivity |
| k_1, k_2, k_3 | <i>a-priori</i> | min^{-1} | C-peptide transport rates |

Table 4.3 Exogenous input variables to the pharmacodynamics/kinetics (ICING) models.

| Variable | Unit | Description |
|----------|------------------------|-----------------------------------|
| P | mmol.min^{-1} | Oral glucose input rate (enteral) |
| U_{ex} | mU.min^{-1} | Exogenous insulin input rate |

In the original work of Lotz (2007), V_G is also identified, while n_K , k_I , k_2 and k_3 are calculated *a-priori* via functions of height, weight, gender and age (Van Cauter *et al.* 1992). Lotz (2007) also used different volumes for plasma and interstitial insulin distribution. However, variance in these values did not impact participants' pharmacokinetics significantly, and were therefore fixed at generic population values for this study.

4.2.4 Grid Analysis of α_G and n_I

As α_G and n_I contribute significantly to the modeled values of Q , both parameters play a critical role in that they link this unmeasurable concentration to the measurable concentrations and compartments for I and G . However, these two parameters cannot be uniquely identified without measurements being available in Q . In reality, the dynamic response of Q is effectively unmeasurable (Duckworth *et al.* 1998; Sherwin *et al.* 1974).

This section analyses the relationship of α_G and n_I parameter values using a grid search method to study their influence on S_I . The analysis range for n_I is [0.001, 0.065] (Duckworth and Kitabchi 1981; Nestler *et al.* 1988), and for α_G is [0, 0.1] (Natali *et al.* 2000; Prigeon *et al.* 1996; Transberg *et al.* 1981). These ranges cover the physiological ranges reported in literature, where the boundaries are beyond reported physiological maximum and minimum levels (Duckworth and Kitabchi 1981; Natali *et al.* 2000; Nestler *et al.* 1988; Prigeon *et al.* 1996; Transberg *et al.* 1981).

Specifically, the variations in model-based S_I are examined across the grid space of α_G and n_I with the resulting intra-patient repeatability in insulin sensitivity as the optimised metric. An

insulin sensitivity test producing the least variation in an individual over multiple tests is usually considered to be more accurate. In addition, limiting intra-patient repeatability in modelling the DISST to within its reported $\sim 11\%$ CV (Lotz *et al.* 2008) ensures the analysis does not over- or under- fit the data.

In particular, it assumes the individual is effectively the same to within test repeatability between tests, which is both realistic and the best assumption available to ground this analysis. The intra-patient variability in S_I is defined:

$$V_{S_I} = \frac{\sum |S_{I_{1..n}} - \bar{S}_I|}{\sum S_{I_{1..n}}} \quad 4.6$$

where n is the number of tests conducted on a single patient.

4.3 Results and discussion

4.3.1 Fitting and grid-search results

The fitted S_I variance for each subject in the parameter space of $\alpha_G = 0 \rightarrow 0.1$ and $n_I = 0.001 \rightarrow 0.065$ generally decreases with increasing n_I , and to a lesser degree, decreasing α_G . The overall variation in S_I in the parameter space across the 18 subjects can be seen in Figure 4.1. The intra-participant variation in S_I is generally low within the parameter space studied, as shown in Figure 4.1(A). The degree of variation is comparable to that for the model-based S_I from the original pilot study of Lotz (2007), which reported intra-participant variation generally of 11%. The darker regions in Figure 4.1(B) are parameter values producing tighter inter-quartile spread of S_I variability across all subjects. The darker areas are similar to areas of low median variability from all patients in Figure 4.1(A). Figure 4.1(C) effectively shows that low α_G and low n_I results in more cohort outliers with large intra-patient variability.

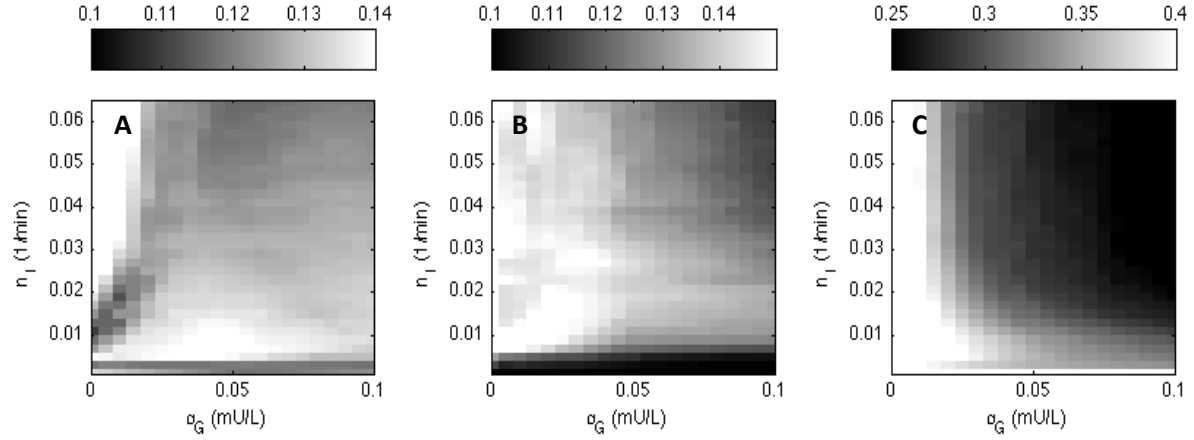


Figure 4.1 Variation of model-based S_I . Color bars are the level of variations. Darker areas of least intra-participant mean S_I variability from different DISST tests. (A) Median variability from all participants. (B) IQR of mean variability amongst participants. (C) 90% CI in variability amongst participants

Per-patient, the spread of S_I variability across the 17 patients can be seen in Figure 4.2(A). The combination $[\alpha_G, n_I] = [0, 0.049]$ represents the population values from the original DISST model Lotz *et al.* (2008). The model-based S_I using $[\alpha_G, n_I] = [0, 0.049]$ in this study correlated well to the S_I calculated using the original DISST model where more parameters are patient-specific (Lotz *et al.* 2008) showing the effect of these changes in assumptions. The correlation of S_I from the model in Equations 4.1-4.5 with these parameters ($[\alpha_G, n_I] = [0, 0.049]$) to the S_I values from the DISST pilot (Lotz *et al.* 2010) and when fit to EIC data (Lotz 2007) is a combined $R=0.93$. This high correlation suggests that model accuracy is not compromised by adapting population values in parameters which involved patient-specific calculation in the original DISST model. The correlation decreases for the other two combinations shown in Figure 4.2(A), where $R=0.85$ when $[\alpha_G, n_I] = [0.05, 0.055]$ is the ‘best fitted’ model-based parameters and $R=0.7$ when $[\alpha_G, n_I] = [0.015, 0.003]$ are the values found for a critically ill cohort model-based parameters (Lin *et al.* 2011).

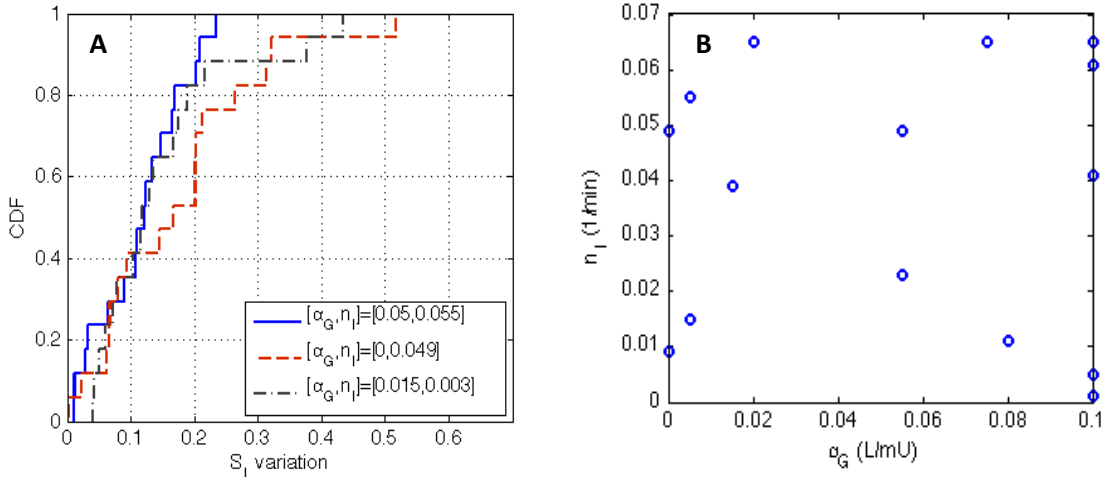


Figure 4.2 (A) Cumulative distribution of patient S_I variability at different α_G and n_I values. (B) α_G and n_I values for the lowest S_I variability in each patient.

The combinations of α_G and n_I producing the lowest intra-patient S_I variability for each patient can be seen in Figure 4.2(B). The best combinations from each of the 17 patients are scattered widely over the parameter space. This scatter may be an indication that these parameters have significant inter-patient variability and patient-specificity in a case with limited structural model identifiability.

The level of saturation in insulin-mediated glucose removal for a given insulin dose has been reported across a wide range. The plasma insulin level at half maximal action of glucose removal has been reported to be between 50-1000 mU.L⁻¹ a range of 20 times. These results are effectively equivalent to α_G between 0.04 and near zero (Docherty *et al.* 2010; Natali *et al.* 2000; Nestler *et al.* 1988; Prigeon *et al.* 1996; Rizza *et al.* 1981; Transberg *et al.* 1981). The saturation in insulin-mediated glucose removal may not simply be due to the number of available receptors. A delay in insulin transportation to the skeletal muscle in addition to transport delay (n_I), common in insulin resistant individuals, would also be seen as saturation in insulin-mediated glucose removal (Barrett *et al.* 2009; Prigeon *et al.* 1996). In addition, the dynamic response of endogenous glucose production to the insulin and blood glucose is causing instability to the model assumption depending on levels of nutrition and assumptions about the role of S_I (Pretty 2012). Thus, the underestimation of endogenous glucose production will also cause glucose

removal to appear slower, effectively adding to the apparent saturation effect in the context of the model defines and its underlying assumptions.

Although the combination of $[\alpha_G, n_I] = [0.015, 0.003]$ limited the variability of intra-patient S_I over the cohort, the resulting S_I is almost equally high for all patients, losing its diagnostic value in insulin resistance screening. The correlation to the original DISST S_I dropped significantly to $R=0.70$. The same results are found when extreme values of α_G are used. The correlation dropped to $R=0.78$ when $[\alpha_G, n_I] = [0.1, 0.065]$. However, the decrease in R value is not as significant as lowering n_I .

The combination $[\alpha_G, n_I] = [0.05, 0.055]$ appears to deliver both good intra-patient variability, while maintaining good diagnostic accuracy. This set of parameter values produced low median S_I variation amongst all subjects where the inter-quartile range is also tight, as seen in Figure 4.1, with median 12%, [IQR: 11, 14]%. The correlation to the original DISST S_I is $R=0.85$ and the median value of 12% is slightly higher than to the reported value 11% of Lotz *et al.* (2008) in its Monte Carlo analysis of the DISST. It is thus at, or near, the expected minimum value. More importantly, the identified S_I also follows the same trend as the original DISST S_I and identified patients with impaired glucose tolerance with similar accuracy ensuring clinical efficacy as well as further form of validation.

A typical DISST test response from a patient is shown in Figure 4.3. The model fits to plasma insulin measurements using different parameter values α_G and n_I are effectively equally good across the physiological parameter space. Therefore, patient-specific α_G and n_I cannot be identified simultaneously with n_L and x_L given that plasma insulin levels are the only measurements available. The effect of α_G and n_I on the shape of insulin at the receptor level (i.e. the *effective* insulin for glucose removal) can be seen in Figure 4.3. Within the physiological range, larger α_G results in a near uniform shift in the level of receptor bound insulin, whereas smaller n_I causes the shape of receptor bound insulin to be flatter with a delay in peak time.

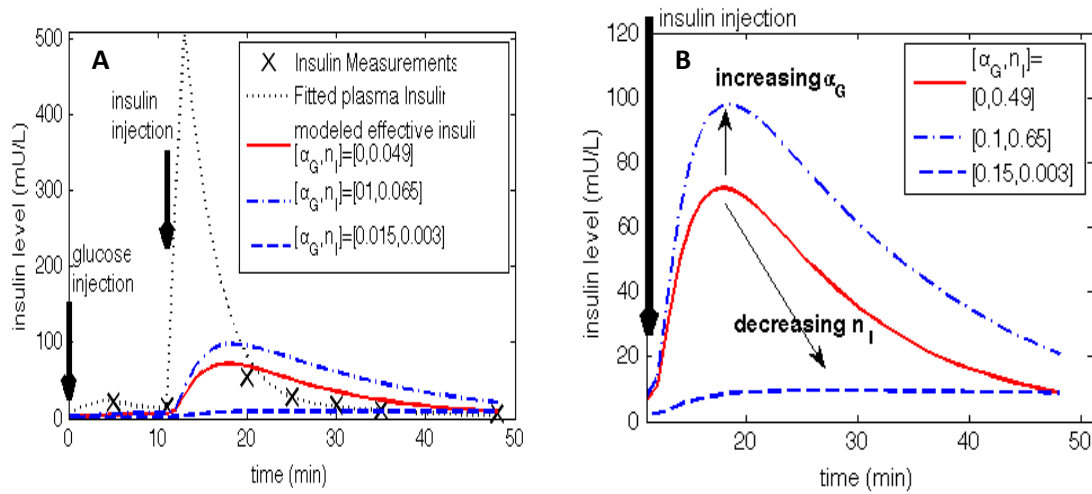


Figure 4.3 Typical DISST (Dynamic Insulin Sensitivity and Secretion Test) test response (A) Plasma and effective insulin. Subfigure (B) focuses on the shape of effective (receptor bound) insulin with different $[\alpha_G, n_I]$.

The level of modeled α_G has been found to have a magnified effect in the S_I of critically ill patients receiving intensive insulin therapy (Chase *et al.* 2004). It can be seen in Figure 4.3(B), varying α_G shifts the magnitude of the modeled “effective” insulin without influencing the time of peak action. Therefore, α_G does not impact the identified value of S_I as much as n_I . In the 17 subjects evaluated in this study, including the saturation parameter α_G enhanced the repeatability of the modeled S_I within a patient, as this eliminates the outlying large variability seen in the low α_G region in Figure 4.1(C).

The level of n_I has both a magnification effect as well as phasing effect on the shape of effective insulin. When the level of n_I decreases, less insulin is able to reach interstitium before being cleared from plasma by the liver and kidneys. The value for n_I in the original DISST model was calculated using models of C-peptide pharmacokinetics (Van Cauter *et al.* 1992). Using these equations, the values of n_I amongst the 17 subjects in this study range between 0.048–0.050. Details for this calculation can be found in Van Cauter *et al.* (1992) and Lotz (2007). A population n_I value of 0.055 is found to provide good intra-patient repeatability in S_I and is in the region found by Van Cauter *et al.* (1992).

4.3.2 Validation and relative residual analysis

In the validation study of the ICING glucose-insulin model, using clinical data from critically ill patients receiving intensive insulin therapy, n_I was found to be very low at 0.003 min^{-1} (Lin *et al.* 2011). This outcome may indicate significant impaired trans-capillary transport for patients who are critically ill. In particular, sepsis causes a dysfunction in mirco-circulation as well as cell metabolism, and is a condition that is prevalent in critical care (Abraham and Singer 2007). However, Pretty (2012) found that the insulin kinetics parameters are $n_I = n_C = 0.006 \text{ min}^{-1}$, which is double the values of the Lin *et al.* (2011) study. These values were optimal in minimising the sum of absolute errors between simulated and measured interstitial insulin concentration. Nevertheless, the level of saturation in insulin-mediated glucose removal is assumed to be constant and unaffected with this new finding. Since these parameters are population constants, they are unlikely to alter the overall variability of S_I , just its relative value.

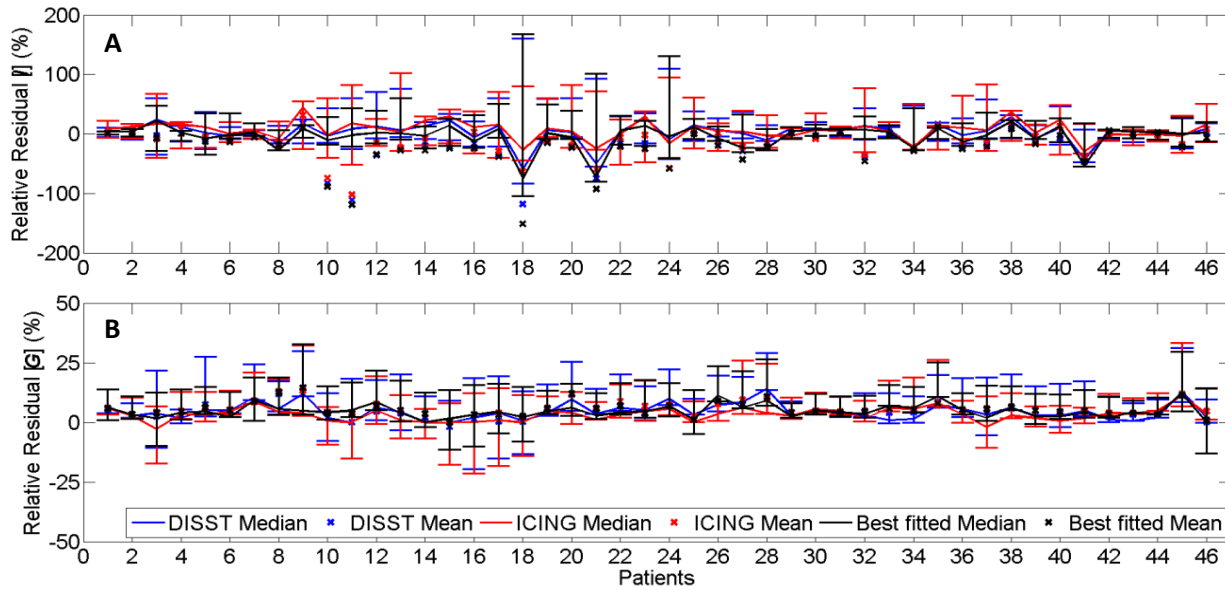


Figure 4.4 Relative residual (median,IQR) per-patient with mean between measured I (A) and G (B) data at different $[\alpha_G, n_I]$ responses based on the model.

This study used data from the DISST and an adapted version of its model of the glucose-insulin pharmacodynamics model. This latest glucose pharmacodynamics model (Pretty *et al.* 2012) is currently being used in the Christchurch Hospital Intensive Care Unit (Evans *et al.* 2011; Fisk *et al.* 2012). The main advantage of using this simple model over a multi-compartmental model is

the model identifiability using limited plasma samples, while capturing and accounting the dominant dynamics of plasma glucose at cohort level. This model has performed well in a variety of insulin and nutrition based glycaemic control trials (Chase *et al.* 2011a; Evans *et al.* 2011; Fisk *et al.* 2012). The trade-offs between the improved structural identifiability of a simplified model and potential fitting errors introduced by simplification of the model are insignificant as the relative residual observed in fitting G responses for each patient is comparatively small as shown in Figure 4.4(B).

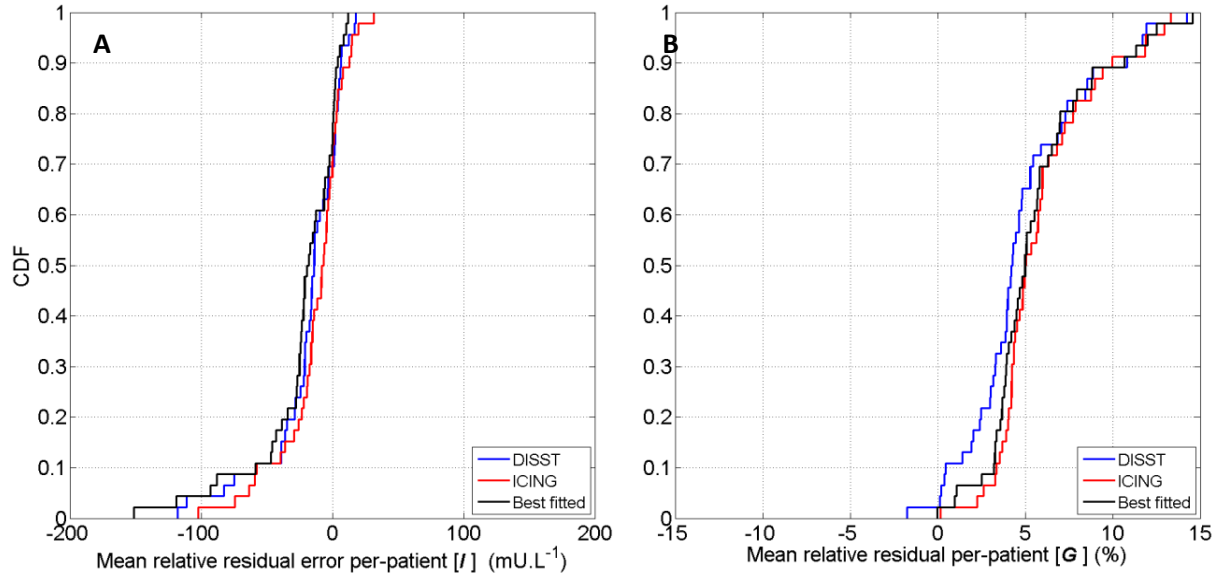


Figure 4.5 Cumulative distribution of mean relative residual, I (A) and G (B) for three different cohort parameters of $[\alpha_G, n_I]$.

Larger population mean relative residuals in I (Table 4.4) indicate high inter-patient variability in modelling and fitting plasma insulin based on measured insulin data as shown in Figure 4.4(A). Based only on population parameters of $[\alpha_G, n_I]$, the model-based compartment is unable to capture the underlying dynamics behind it. However, the modeled dynamics appear similar as Figure 4.2 clearly shows that low variability in model-based S_I is achievable via participant-specific parameters identification for $[\alpha_G, n_I]$. Comparatively, median relative residual for I is higher than G as in Figure 4.5. Importantly, the 90% confidence interval of the residuals in Figure 4.5 are all at or within measurement error for their respective species.

For cases of poor fit, it may be suspected that a contributor to these poor fits is caused by differences in incretin effects for each subject. Incretins affect the endogenous insulin secretion/production (U_{en}) response to change in glucose concentration. Previous results from Despopoulos and Silbernagl (2003) verified C-peptide, just like insulin, is produced by the β -cells located in the islets of Langerhans (pancreas). However, C-peptide is only cleared by renal and thus, has longer half-life in comparison to insulin (Guyton and Hall 2000). To this point, plasma C-peptide concentrations are still the best indication of pancreatic insulin secretion due to equimolar secretion and longer half-life.

Table 4.4 Population mean relative residual summary at different $[\alpha_G, n_I]$ values.

| Cohort $[\alpha_G, n_I]$ | Mean Relative Residual (%) | | Median [IQR] | | 90% CI | |
|----------------------------------|----------------------------|--|--------------|------------|---------|-----------|
| | | | G | I | G | I |
| DISST [0,0.049] | | | 4 [3,7]% | 5 [-24,2]% | [0,12]% | [-88,13]% |
| ICING (ICU) [0.015,0.003] | | | 3 [4,7]% | 6 [-19,1]% | [3,12]% | [-65,16]% |
| Best fitted [0.05,0.055] | | | 4 [4,7]% | 2 [-27,0]% | [1,12]% | [-98,9]% |

Fairly good fit in modeled I is obtained by grid search analysis with median at 2% of mean relative residual when $\alpha_G=0.05$ and $n_I=0.055$. However, the model still delivers good fit with other combinations of $[\alpha_G, n_I]$ at a cohort level. Thus, the pharmacodynamics/kinetics model can tolerate a range of any combinations of $[\alpha_G, n_I]$ that covers most physiological ranges reported in literature indicating the aforementioned lack of structural identifiability used to justify the grid search used. In particular, parameter identification trade-off between these parameters causes unique identification to be impossible. The inability to discover this relationship at an inter-patient level suggested that there are still underlying effects that contribute to this uncertainty. These effects might be from human physiology or/and in modelling methodology (Chase *et al.* 2011b; Cobelli *et al.* 2007; Docherty *et al.* 2011).

4.4 Summary

This chapter presented an independent examination of the model roles of α_G and n_I for describing insulin and glucose pharmacodynamics and pharmacokinetics. Interstitial insulin kinetics and their influence on model-based insulin sensitivity observation was analysed using data from the clinical pilot study of the dynamic insulin sensitivity and secretion (DISST) test and the glucose-insulin PK-PD models. From these inputs, a model of interstitial insulin dose-response that best links insulin action in plasma to response in blood glucose levels was developed. The critical parameters influencing interstitial insulin pharmacokinetics (PKs) are saturation in insulin receptor binding (α_G) and the plasma-interstitium diffusion rate (n_I). The optimal population values for these parameters are found to be $[\alpha_G, n_I]=[0.05,0.055]$.

However, increasing the number of identified participant-specific parameters did not necessarily improve model accuracy, as inter-patient fitting error in plasma insulin was quite high compared to fitting error in plasma glucose.

The intra-patient repeatability of S_I and its link to interstitial insulin action is studied in 17 patients. Very low values of insulin receptor saturation α_G and very low values of plasma-interstitial insulin diffusion n_I are found to produce the most intra-patient variability in S_I . A model accounting for insulin receptor saturation enhanced the repeatability in S_I . A cohort of patients with mixed levels of insulin resistance will also further validate the accuracy of the model-based S_I and may provide a better understanding of the contributing factors of insulin resistance. “Customising” patient-specific identification on α_G and n_I might suggest the best repeatability in S_I . This might also increase model fitting accuracy of plasma insulin and interstitial insulin.

However, more test data from larger cohorts will enable a more in-depth study of saturation in mediated glucose removal and plasma-interstitium insulin diffusion, or the actions of insulin in the interstitium in general. Once pharmacokinetics and pharmacodynamics of plasma insulin and interstitial insulin are better measured and understood, it may refine the understanding of endogenous insulin production covering the incretin effects.

Chapter 5. Incretin Effects of Critically Ill Patients

Critically ill patients are regularly fed via constant enteral (EN) nutrition infusions. The impact of incretin effects on endogenous insulin concentration in this cohort remains unclear. This chapter investigates whether EN-driven incretin effects should alter insulin and glycemic management in critically ill patients.

5.1 Introduction

Critically ill patients exhibit increased gluconeogenesis, reduced insulin secretion and increased insulin resistance, resulting in hyperglycaemia, increased complications and increased risk of mortality (Capes *et al.* 2000; Van den Berghe *et al.* 2001). Studies show that glycaemic control can mitigate these outcomes (Krinsley 2004; Van den Berghe *et al.* 2001). However, intensive insulin therapy can also lead to increased hypoglycaemia and mortality (Bagshaw *et al.* 2009b; Chase *et al.* 2011a). Variable patient-specific levels of endogenous insulin secretion play a role in this variability, particularly in early, acute phases of care (Bagshaw *et al.* 2009a).

In addition, the route used for the provision of nutrition can also influence the effect of intensive insulin therapy. A recent meta-analysis (Marik and Preiser 2010) demonstrated that intensive insulin therapy was not associated with an improved outcome when enteral nutrition was used as the predominant source of calories. This finding is consistent with the presence of an incretin effect, i.e. the stimulation of endogenous insulin secretion by enteral feeding.

The incretin effect plays an important role in glucose homeostasis in healthy subjects (Schirra *et al.* 1996). In particular, as noted in Chapter 2, incretin enhances the postprandial appearance of endogenous insulin (Holst and Gromada 2004; Muscelli *et al.* 2008; Nauck *et al.* 1986; Vilsbøll and Holst 2004). The underlying mechanisms involve the release of gastrointestinal hormones gastric inhibitory peptide (GIP) and glucagon-like peptide-1 (GLP-1), which are released from the intestinal mucosa when glucose is ingested (Faber *et al.* 1979; Holst and Gromada 2004; McIntyre *et al.* 1965; Nauck *et al.* 1986; Perley and Kipnis 1967; Schirra *et al.* 1996). As a result, insulin secretion is enhanced in excess of what would have been released if the glucose

were administered parenterally via intravenous lines (Dalla Man *et al.* 2006; McIntyre *et al.* 1965; Perley and Kipnis 1967). More specifically, studies have shown that the incretin effect can enhance the insulin response to oral glucose by 50–70% in comparison to an equivalent IV dose (Nauck *et al.* 1986; Whyte *et al.* 2010).

Although many incretin effect studies have addressed the distinct physiology of diabetic and non-diabetic individuals (Dalla Man *et al.* 2010; Holst and Gromada 2004; Muscelli *et al.* 2008; Nauck *et al.* 2004; Perley and Kipnis 1967), the incretin effects has not been observed in a critically ill cohort. Critically ill patients are typically fed with constant EN infusions. Hence, it is possible that there is no incretin effect observable in these patients, who otherwise display significantly enhanced endogenous insulin secretion (Pretty 2012; Whyte *et al.* 2010). Equally, their highly counter-regulated state and wide range of insulin secretion rate (Capes *et al.* 2000) may result in a blunting of this responses, as seen similarly in individuals with type 2 diabetes (Muscelli *et al.* 2008).

This analysis uses dense clinical data and a model-based analysis to observe incretin effects by tracking the identified model-based insulin sensitivity (S_I) in a cohort of critically ill patients. It is hypothesised that the identified S_I would decrease during interruptions of EN and would increase when EN is resumed, where, for short periods around transition, the true patient S_I would be assumed constant. Thus, changes in modeled S_I given the fixed assumed endogenous secretion by the model would support the presence of an EN-related incretin effect in the population of non-diabetic, critically ill patients studied.

5.2 Subjects and Methods

5.2.1 Patient cohort

Data was obtained earlier from the Specialized Relative Insulin Nutrition Titration (SPRINT) study (Chase *et al.* 2008). Blood glucose concentration (G) and EN nutrition data from 371 critically ill patients on SPRINT protocol were used. These 371 patients were undergoing SPRINT tight glycaemic control (TGC) where insulin and nutrition are given in balance based on the response to the prior insulin and nutrition intervention (Chase *et al.* 2010; Lonergan *et al.*

2006). Hence, the protocol prevents hyperglycaemia by matching the nutrition and exogenous insulin given to the body's patient-specific ability to utilise them.

For this study, patient inclusion required a minimum of 10 hours of EN feeding, followed by at least 7 hours with EN off, and then at least 5 hours of resumed feeding. Hence, only 52 of 371 SPRINT patients' were eligible for analysis. Patients with diabetes (N=64) were excluded due to irregularity of the incretin effect that common only occurs in diabetes (Muscelli *et al.* 2008; Nauck *et al.* 2004; Nauck *et al.* 1986). Another 255 non-diabetic patients were excluded as they did not have a period of zero EN input. The clinical details of this selected cohort (N=52), including baseline variables, Acute Physiology and Chronic Health Evaluation (APACHE) II scores and APACHE III diagnosis codes are summarised in Table 5.1. Data from the excluded non-diabetic patients (N=255) is included for comparison.

Table 5.1 SPRINT cohort baseline variables summary. Data expressed as median [IQR] (APACHE = Acute Physiology and Chronic Health Evaluation).

| Patient Data from SPRINT | | | |
|---------------------------------------|-------------------------------|-----------------|----------|
| | Excluded, non-Diabetic cohort | Included cohort | |
| | N=255 | *N=52 | |
| Age (years) | 65 [51-74] | 65 [49-72] | $p=0.86$ |
| % Male | 70% | 67% | $p=0.67$ |
| APACHE II score | 18 [14-23] | 19 [17-28] | $p=0.07$ |
| APACHE III Diagnosis | Number of cohort (%) | | |
| Cardiovascular | 38 (15%) | 5 (10%) | |
| Respiratory | 64 (25%) | 8 (15%) | |
| Pancreatitis | 11 (4%) | 7 (13%) | |
| Neurological | 21 (8%) | 6 (12%) | |
| Trauma | 38 (15%) | 6 (12%) | |
| Sepsis | 12 (5%) | 7 (13%) | |
| Other (Renal, metabolic, orthopaedic) | 71 (28%) | 13 (25%) | |

*N=52 are the patient data used in this study. Note N=255 and N=52 excluded diabetic data. p -values based on ranksum test.

5.2.2 EN feeding criterion

The transition off EN (ON/OFF) is defined when EN nutrition given to the patient is stopped, while the (OFF/ON) transition denotes when EN is recommenced, as illustrated in Figure 5.1. These times are known to within a maximum of ± 30 min from patient's chart data. The glucose input from EN infusion varies from 0 to $1.65 \text{ mmol} \cdot \text{min}^{-1}$ where the range of patient-specific goal nutrition rates is $0.4\text{--}0.8 \text{ mmol} \cdot \text{min}^{-1}$ (Chase *et al.* 2008). The EN formulation was either from Glucerna[®] 1.2 CAL (Abbott 2005) or RESOURCE[®] Diabetic (Novartis 2005). The specific nutrition compositions are given in Table 5.2.

Table 5.2 Enteral nutrition composition based on GLUCERNA[®] 1.2 CAL and RESOURCE[®] Diabetic (Novartis).

| | Glucerna [®] 1.2 CAL (Abbott) | RESOURCE [®] Diabetic (Novartis) |
|----------------------|---|--|
| Carbohydrate (Fiber) | 35 % (16 g/1000 mL) | 36 % (12 g/1000 mL) |
| Protein | 20 % | 24 % |
| Fat | 45 % | 40 % |

The ON/OFF and OFF/ON EN transitions are defined:

EN (ON/OFF) transition: Transition between minimum of 10 hours on EN feeding and followed by at least 7 hours off EN feeding.

EN (OFF/ON) transition: Transition between minimum of 7 hours off EN feeding and followed by at least 5 hours of resumed EN feeding.

where Figure 5.1 illustrates an example of the EN nutrition input rate during SPRINT TGC protocol. The EN feeding is stopped at $t=118$ hours and EN feeding is resumed after 7 hours off feed at $t=126$ hours.

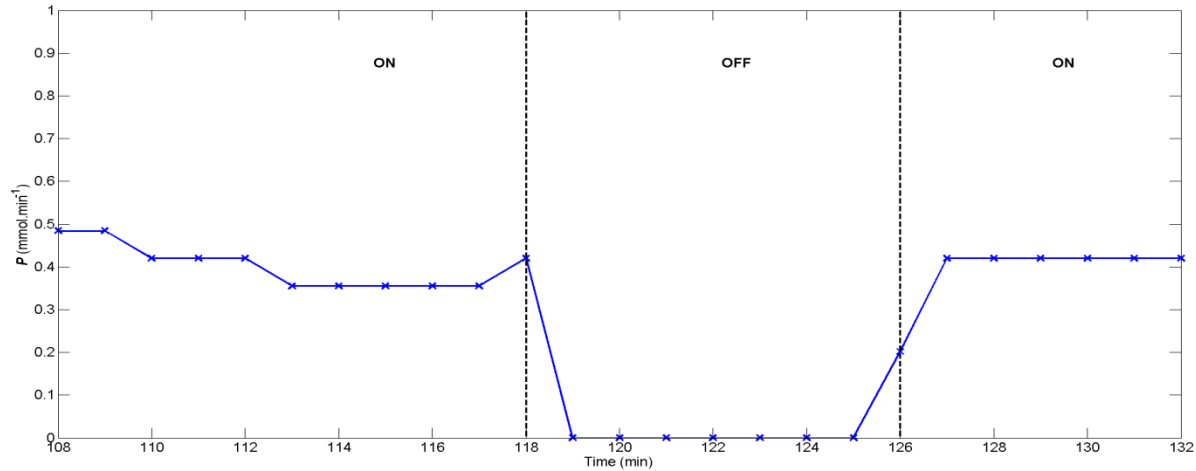


Figure 5.1 The ON/OFF and OFF/ON EN transitions. ($P(t)$) showing rate ($P(t)$) on the y-axis and time (hours) along x-axis.

All critically ill patients that underwent SPRINT were continuously infused with EN feeding in balance with the insulin input to prevent hypoglycaemia and/or hyperglycaemia. SPRINT controls both insulin and nutrition inputs. Specifically, SPRINT modulates nutritional intake between 30–100% of patient-specific goal feed rate based on ACCP/SCCM guidelines (Cerra *et al.* 1997). SPRINT also specifies only low-carbohydrate EN nutrition formula with 35–40% carbohydrate content, as shown in Table 5.2, unless clinically specified otherwise in rare cases. Importantly, SPRINT determines both insulin and nutrition interventions 1-2 hours based on estimated insulin sensitivity of the patient in response to the prior interventions, rather than depending on blood glucose concentrations or/and changes alone. Hence, insulin and nutrition are given in accordance with explicit knowledge of carbohydrate intake, which is unique to this protocol (Suhaimi *et al.* 2010). The overall mechanism thus matches the nutrition and exogenous insulin infused to the body's patient-specific ability to adequately utilise them, which resulted in better control and a reduction in hypoglycaemia (Chase *et al.* 2008).

5.2.3 Identification of S_I

Patient-specific S_I is identified assuming constant model-based endogenous insulin secretion because insulin secretion cannot be directly measured at bedside in real-time to guide therapy. This assumption is used to measure the presence of an incretin effect. S_I is identified hourly using the iterative integral method (IIM) (Docherty *et al.* 2010) and validated clinical data from SPRINT TGC protocol.

The clinically validated Intensive Control Insulin-Nutrition-Glucose (ICING) model Equations 5.1-5.6 presented by Lin *et al.* (2011) is used to identify hourly S_I (L.mU⁻¹.min⁻¹) from each patient's clinical data:

$$\dot{G} = -p_G G - S_I G \frac{Q}{1 + \alpha_G Q} + \frac{P + EGP - CNS}{V_G} \quad 5.1$$

$$\dot{Q} = n_I(I - Q) - n_C \frac{Q}{1 + \alpha_G Q} \quad 5.2$$

$$\dot{I} = -n_k I - n_L \frac{I}{1 + \alpha_I I} - n_I(I - Q) + \frac{U_{ex}}{V_I} + (1 - x_L) \frac{U_{en}}{V_I} \quad 5.3$$

$$\dot{P}_1 = -d_1 P_1 + D \quad 5.4$$

$$\dot{P}_2 = -\min(d_2 P_2, P_{max}) + d_1 P_1 \quad 5.5$$

$$P = \min(d_2 P_2, P_{max}) + PN \quad 5.6$$

All ICING model parameters are based on population values of critically ill patients (Lin *et al.* 2011), which are defined in Tables 5.3 and 5.4.

Table 5.3 Parameter (stimulated) values and descriptions for the ICING model.

| Variable | Value | Unit | Description |
|----------|------------|------------------------|---|
| G | stimulated | mmol.L ⁻¹ | Blood glucose concentration |
| Q | stimulated | mU.L ⁻¹ | Interstitial insulin concentration |
| I | stimulated | mU.L ⁻¹ | Plasma insulin concentration |
| P_1 | stimulated | mmol | Glucose appearance in the stomach |
| P_2 | stimulated | mmol | Glucose appearance in the gut |
| P | stimulated | mmol.min ⁻¹ | Glucose appearance in blood from EN nutrition |

Table 5.4 Parameter values and descriptions for the ICING model.

| | | | |
|------------|-----------------|-------------------------------|---|
| p_G | 0.006 | min^{-1} | Non-insulin mediated glucose removal |
| EGP | 1.16 | $\text{mmol}.\text{min}^{-1}$ | Endogenous glucose production rates |
| CNS | 0.3 | $\text{mmol}.\text{min}^{-1}$ | Central nervous system glucose uptake |
| V_G | 13.3 | L | Plasma glucose distribution volume |
| V_I | 4.0 | L | Plasma and interstitial insulin distribution volume |
| α_G | 0.0154 | $\text{L}.\text{mU}^{-1}$ | Insulin binding saturation parameter |
| α_I | 0.0017 | $\text{L}.\text{mU}^{-1}$ | Hepatic insulin clearance saturation parameter |
| n_I | 0.006 | min^{-1} | Trans-endothelial diffusion rate |
| n_C | 0.006 | min^{-1} | Interstitial insulin degradation rate |
| n_K | 0.0542 | min^{-1} | Renal insulin clearance rate |
| n_L | 0.1578 | min^{-1} | Hepatic insulin clearance rate |
| x_L | 0.67 | 1 | Fractional first-pass hepatic insulin extraction |
| d_1 | 0.0347 | min^{-1} | Glucose transport rate from stomach to gut |
| d_2 | 0.0069 | min^{-1} | Glucose transport rate from gut to plasma |
| P_{max} | 6.11 | $\text{mmol}.\text{min}^{-1}$ | Maximum glucose flux from gut to plasma |
| U_{en} | 1000 | $\text{mU}.\text{min}^{-1}$ | Endogenous (basal) insulin secretion |
| PN | <i>a-priori</i> | $\text{mmol}.\text{min}^{-1}$ | Parenteral nutrition input rate (Intravenous) |
| D | <i>a-priori</i> | $\text{mmol}.\text{min}^{-1}$ | Enteral nutrition (dextrose) input rate |
| U_{ex} | <i>a-priori</i> | $\text{mU}.\text{min}^{-1}$ | Intravenous insulin input rate |

5.2.4 Data analysis and observation of incretin effect

A notable reduction in model-based S_I after the ON/OFF transition implies an un-modeled decrease in the rate of endogenous insulin secretion due to the loss of incretin effects when feed is stopped rather than a true reduction of physiological S_I . In contrast, an increase in observed S_I implies an incretin effect at the OFF/ON transition when the feed is commenced. The S_I change (ΔS_I) across the ON/OFF and OFF/ON transitions indicates an incretin effect as its stoppage for this analysis. ΔS_I was calculated as:

$$\Delta S_I = 2 \frac{S_{I(t)} - S_{I0}}{S_{I(t)} + S_{I0}} \quad 5.7$$

The blood glucose changes, ΔG , over these periods were also calculated similarly.

The analysis uses a 3-hour moving average to reduce the effect of measurement error, noise and the influence of transient effects caused by cohort-constant assumption of these model terms. S_I profiles are derived over periods starting 3 hours before a transition until 5 hours after the transition. The 5-hour limit allows full gut emptying after ON/OFF transition or full resumption of the effect EN after the OFF/ON transition. Between these times, an incretin effect would show a steady biased shift in S_I , if it exists. Results are illustrated via Bland–Altman plots, while Wilcoxon rank sum tests (p -values) are used to compare distributions and the significance of any shift in $S_I(t)$ over the cohort.

The changes in plasma insulin via insulin secretion and activation by the liver were assumed to be observable through changes in measured S_I (Lin *et al.* 2011). The variability observed was outside the normal variation, which is centred around zero (Lin *et al.* 2008). Thus, any incretin effect is assessed using the shift in S_I after EN feed transitions, as a surrogate, rather than from a direct insulin measurement. It could be concluded that insulin sensitivity changes are a more efficient indicator for post-hepatic endogenous insulin appearance (Nauck *et al.* 1986), given the two possible outcome causes noted

5.3 Results and Discussion

5.3.1 S_I correlations over EN transitions

S_I correlations over the ON/OFF ($r=0.49$, median=-36%, $p=0.0001$) and OFF/ON ($R=0.60$, median=31%, $p=0.03$) transitions for $t=4$ hour are shown in Figure 5.2(A and B). The changes of S_I at two EN transitions of ON/OFF in Tables 5.5 and OFF/ON in Table 5.6 are evidenced by the bias about the equality line. Figure 5.2(A and B) shows the diversity of ΔS_I across both transitions.

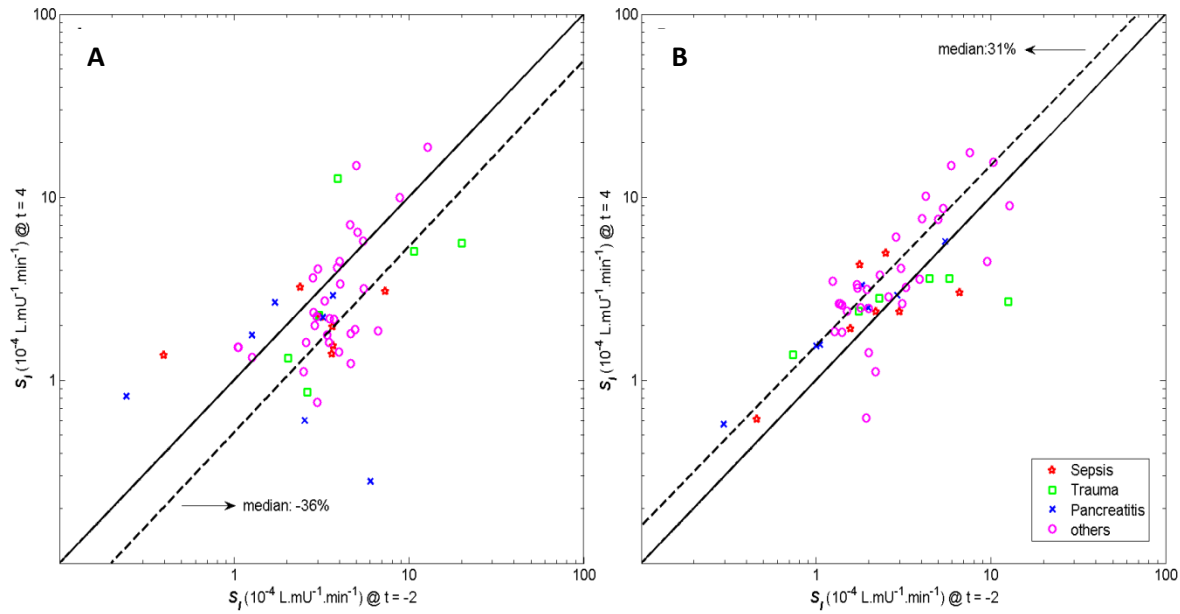


Figure 5.2 The distributions of S_I for two transitions of ON/OFF (A) and OFF/ON (B) EN transitions at the centred time averages of $t=-2$ and $t=4$ hour ($N=52$). (Note the log-scale).

Tight and dense S_I distribution is observed in Figure 5.2(B) at the OFF/ON transition implies that resuming EN nutrition causes measurable changes in insulin secretion in response to EN nutrition input for a majority of the patient studied. While at the ON/OFF transition, the S_I distribution in Figure 5.2(A) around the median line shows a modest number of confounders who behave differently.

Table 5.5 summarises ΔS_I at the ON/OFF transition across the cohort. S_I decreased after the ON/OFF transition until $t=4$ hour, where it settled to a median reduction of -36%. The right-most

column shows the rate of confounders ($\Delta S_I > 0$). This implies that inter-patient or intra-patient variation obscures the observation or that the effect itself if it is not always observable.

Table 5.5 Summary of proportional change of blood glucose, ΔG , and insulin sensitivity, ΔS_I , at ON/OFF feed transition (N=52).

| ON/OFF | | | | | | | |
|--------------|------------------------------|-----------|------------|--------------------------------------|-----------|------------|----------------------|
| t^* (hour) | Blood Glucose (ΔG) | | | Insulin Sensitivity (ΔS_I) | | | |
| | Median | IQR | p -value | Median | IQR | p -value | S_I (\uparrow) |
| -2 | 0% | [0,0]% | 1.0 | 0% | [0,0] | 1.0 | 50% |
| -1 | 0% | [-3,6]% | 1.0 | 0% | [-10,12]% | 0.9 | 50% |
| 0 | -1% | [-9,5]% | 0.7 | -2% | [-16,18]% | 0.7 | 46% |
| 1 | -2% | [-12,8]% | 0.6 | -9% | [-30,23]% | 0.3 | 38% |
| 2 | -1% | [-12,9]% | 0.6 | -10% | [-45,15]% | 0.05 | 27% |
| 3 | -4% | [-15,10]% | 0.5 | -29% | [-59,15]% | 0.01 | 29% |
| 4 | 0% | [-16,17]% | 0.4 | -36% | [-82,24]% | 0.001 | 37% |

*Times are 3-hour averages centred at the time shown, and the feed transition between $t = -1$ and 0. EN feed is stopped anytime between $t = -1$ and $t = 0$.

When EN nutrition stopped, significant ΔS_I was observed from $t=2$ hours ($p < 0.05$). ΔS_I implies that insulin secretion response is decreasing after EN nutrition stopped to maintain normoglycaemia. Importantly, at this state incretins are suppressed which reduces insulin secretion and thus modeled S_I relative to the fixed model-based value assumed. Thus, the model performance in tracking incretin effect via S_I is indistinct at the ON/OFF transition although it is sign that at $t=2$ hours, $\Delta S_I=-10\%$ and $t=4$ hours, $\Delta S_I=-36\%$ with 37% of confounders' exhibiting a confounding increased S_I .

The computed ΔS_I after the OFF/ON transition are shown in Table 5.6. Median ΔS_I increased by 32% [IQR: -1 to 60%] at $t=3$ hour after the OFF/ON EN transition. A slightly stronger incretin effect is observed at OFF/ON feed transition with only 25% of patients confounding expectation.

The insulin response to EN glucose was noticeably enhanced after the OFF/ON transition, most likely as a result of the concomitant actions of incretins and neural responses to EN nutrition. The predominant effect of incretin hormones is to enhance the endogenous insulin secretion that

is triggered when the β -cells are exposed to rapid increases in glucose flux (Cobelli *et al.* 2007). At a cohort level, ΔS_I stabilised at $t=4$ hour after EN transitions as the feed was designed for enhanced glycaemic stability via slow ingestion (Abbott 2005; Novartis 2005). Hence, both transitions should have excited a change, but the OFF/ON transition may have observed better or more rapid change due to the essentially fasted patient state.

Table 5.6 Summary of proportional change of blood glucose, ΔG , and insulin sensitivity, ΔS_I , at OFF/ON feed transition (N=52).

| OFF/ON | | | | | | | |
|--------------|------------------------------|-----------|------------|--------------------------------------|-----------|------------|------------------------|
| t^* (hour) | Blood Glucose (ΔG) | | | Insulin Sensitivity (ΔS_I) | | | |
| | Median | IQR | p -value | Median | IQR | p -value | S_I (\downarrow) |
| -2 | 0% | [0 0]% | 1.0 | 0% | [0 0]% | 1.0 | 50% |
| -1 | -1% | [-6,2]% | 0.6 | 4% | [-13,12]% | 0.9 | 39% |
| 0 | -1% | [-9,6]% | 0.9 | 8% | [-14,23]% | 0.9 | 40% |
| 1 | -3% | [-11,7]% | 0.6 | 14% | [-5,29]% | 0.5 | 33% |
| 2 | -2% | [-10,15]% | 0.9 | 25% | [-10,46]% | 0.3 | 29% |
| 3 | 1% | [-12,15]% | 0.7 | 32% | [-5,53]% | 0.05 | 32% |
| 4 | 6% | [-15,18]% | 0.5 | 31% | [-1,60]% | 0.03 | 25% |

*Times are 3-hour averages centred at the time shown, and the feed transition between $t = -1$ and 0. EN feed is stopped anytime between $t = -1$ and $t = 0$.

Patients were effectively in the fasting state before EN nutrition recommenced between $t=-1$ and 0 hours at the OFF/ON transition. As EN nutrition re-started, modeled S_I increased significantly at $t=3$ to $t=4$ hour ($p < 0.05$) with a 25% confounder rate. Thus, insulin secretion was also increased in response to EN nutrition input via the unsuppressed incretin hormones (Holst and Gromada 2004; Schirra *et al.* 1996; Vilsbøll and Holst 2004) to maintain glucose homeostasis. Overall, the incretin effects are distinctly observable at the ON/OFF transition given ΔS_I rise.

Bland–Altman representations of the ΔS_I changes between $t=-2$ and $t=4$ hours are shown in Figure 5.3 for both the ON/OFF and OFF/ON transitions. Only 3 patients clearly showed high ΔS_I measurements ($>100\%$). These patients were diagnosed with either sepsis or pancreatitis and

are shown with separate markers. Both conditions significantly affect endogenous insulin secretion independently and may well account for these very large changes.

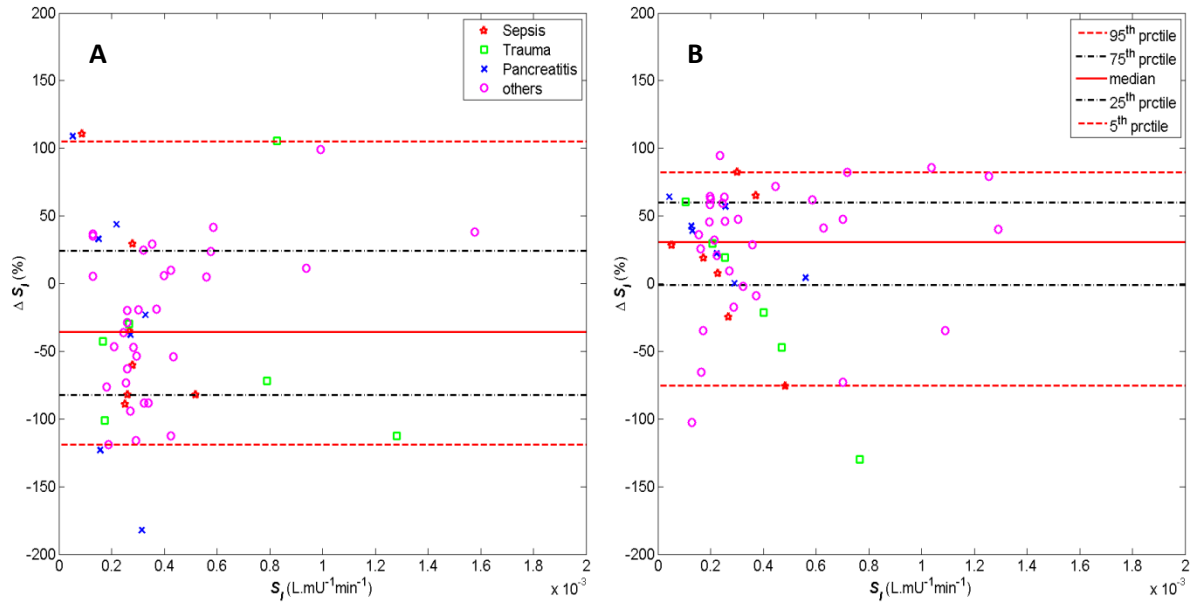


Figure 5.3 The Bland–Altman of the averages of proportional change S_I after the ON/OFF (A) and OFF/ON (B) EN transitions at the centred time averages of $t=-2$ and $t=4$ hour ($N=52$).

The Bland–Altman plots of ΔS_I (Figure 5.3) show that the few larger outliers were diagnosed with chronic diseases that influence the pharmacodynamics of insulin and glucose. Patient with sepsis, trauma and pancreatitis can exhibit more drastic S_I changes (Carlson 2003) due to excessive counter-regulatory and acute immune response, as well as the direct affect on secretion with pancreatitis. Hence, the variability of the results was somewhat expected. The analysis without these 3–5 subjects did not change the overall results.

In addition, studies show that many metabolic abnormalities associated with stress, injury or infections were related to loss of tissue sensitivity to insulin (Carlson 2003). Sepsis, trauma and other clinical states are characterised by a strong counter-regulatory hormone response. These hormone responses are believed to induce insulin resistance *in vivo*, although some clinical studies failed to demonstrate correlations between counter-regulatory hormone response and defective insulin-mediated glucose disposal (Thorell *et al.* 1999). However, the overall evidence is still inconclusive concerning the exact cellular and molecular mechanisms underlying insulin

resistance in critical illness and their relationship to the observed metabolic abnormalities (Thorell *et al.* 1999).

The shifts in G after the ON/OFF and OFF/ON transitions between $t=-2$ and $t=4$ hours are shown in Bland-Altman format in Figure 5.4. The maximal median G shift across the cohort was -4% at the ON/OFF transition and 6% at the OFF/ON transition when $t=3$ and $t=4$ hour respectively. Median difference between these two transitions was approximately -1% at $t=-1$, 1 and 2 hour indicating tight consistent glucose levels across the cohort. The few patients outside the 90% confidence interval (CI) were identified as having pancreatitis or similar disease that significantly affects insulin secretion and thus, potentially, this analysis, as listed previously.

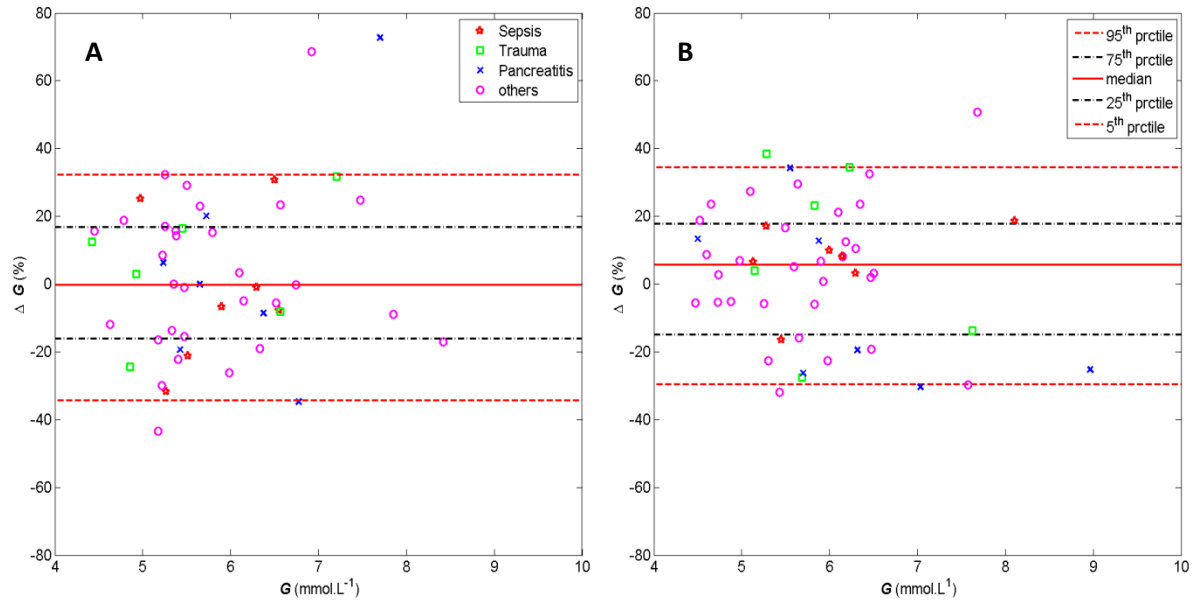


Figure 5.4 The Bland–Altman of the averages of proportional change G after ON/OFF (A) and OFF/ON (B) EN transitions at the centred time averages of $t=-2$ and $t=4$ hour (N=52).

In both cases, G remains effectively constant with only small changes. Hence, the impact of the incretin effect on glycaemic control was quickly accounted for by the SPRINT TGC protocol.

More specifically, ΔG changes were insignificant over these transitions indicating there was no bias to this factor in the model-based analysis. Equally, this model and S_I metric have been clinically validated on independent matched cohorts (Chase *et al.* 2010) in several clinical TGC

studies (Chase *et al.* 2007; Evans *et al.* 2011; Fisk *et al.* 2012; Suhaimi *et al.* 2010) and against the gold-standard EIC (Lotz *et al.* 2008; McAuley *et al.* 2011).

5.3.2 Model fitting

Measured glucose data, EN model input data as well as ICING model fits of G , I , Q , S_I and P are shown in Figure 5.5(A,B and C) for a typical case. The incretin effect, as hypothesised, is observed directly via ΔS_I at the ON/OFF and OFF/ON EN transitions.

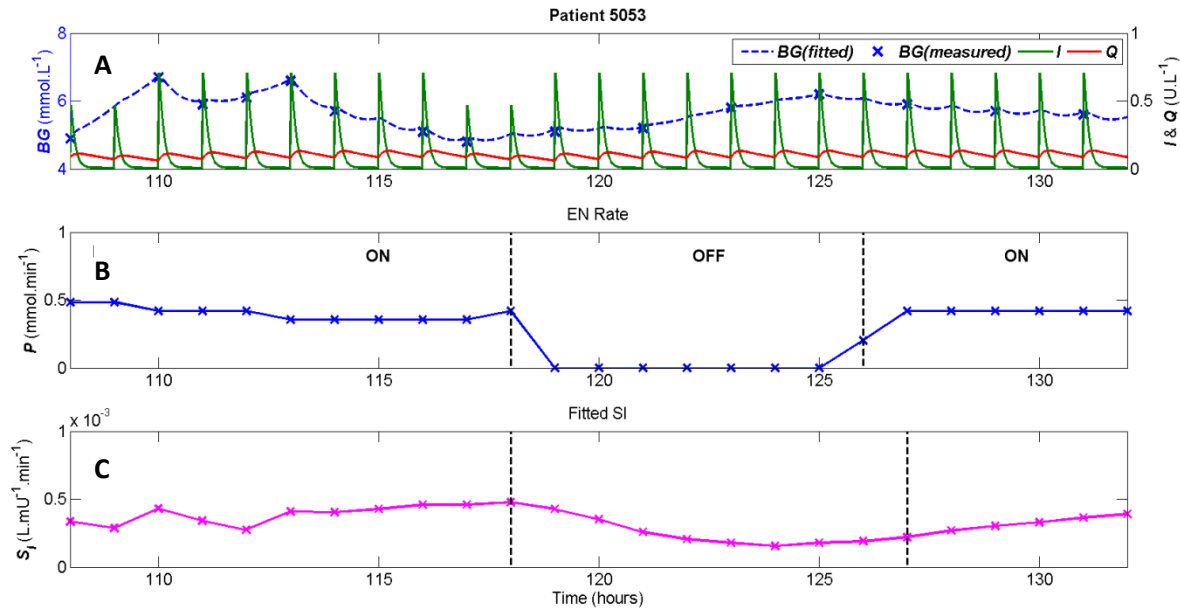


Figure 5.5 A typical patient's fitted profile. (A) Blood glucose, plasma insulin and interstitial insulin fitted profiles. (B) Nutrition (EN) rate (C) time variant insulin sensitivity (S_I).

The ICING model used in this analysis is more explicitly physiological relevant without increasing the number of patient-specific parameters to be identified. More importantly, this approach allows S_I be uniquely identified via patient-specific with limited availability of measured data to 1-2 hourly G measurements. The practical model identifiability is quite strong despite the limited data available and many population assumptions required to uniquely identified S_I (Docherty *et al.* 2011; Lin *et al.* 2011). However, the model structure physiologically relevant and, clinically, the model thus uses and identifies only a single relevant parameter compared to other pharmacokinetics and pharmacodynamics models (Hovorka *et al.* 2004; Parker and Doyle 2001). It is also able to accurately capture the highly dynamic response in critical illness i.e. incretin effects by tracking S_I changes. The low and tightly distributed,

prediction errors reported for this model are within the clinical measurement error of 7–12% (Chase *et al.* 2010; Fisk *et al.* 2012), and signify the model is specifically customised to patient-specific behaviour in the real-time. In addition, the model and the S_I parameters were extremely cross validated in a study by Chase *et al.* (2010).

5.4 Limitations

Incretin effects were observed and isolated in this analysis using EN nutrition changes and the study did not directly measure gastrointestinal hormone (GLP-1 and GIP) concentrations. Thus, the relative insulin secretion across PN/EN feeds must be measured to directly elucidate the incretin effects. However, this analysis provides good evidence for the existence and measurement of these effects, meriting the measurement of these hormones.

5.4.1 PN feeding and U_{en} secretion

This analysis only examined changes in S_I about EN feeding transitions. Limited PN data (N<6 patients) limited observation of the incretin effect to those patients with EN according to its most commonly used definition in comparison to PN. In this study, the SPRINT glycaemic control protocol also modulates enteral dextrose carbohydrate to aid control of hyperglycaemia, rather than the characterising the overall nutritional profile. Hence, a cross-over analysis with PN was not possible. A study conducted by Casaer *et al.* (2011) found that a high glucose loading, via PN, with inadequate glycaemic control is associated with increased morbidity and mortality. Thus an ideal study design for the observation of incretin in critically ill patients would also use PN feeding in a cross-over format. However, this format might have ethical limitations. In contrast, future studies could incorporate direct measurement of the incretin hormones, such as GIP and GLP-1. This approach would also allow direct incorporation and identification of additional incretin hormone-related model parameters, as well as direct measurement of the effect without relying on PN analysis.

It is generally accepted that intravenous parenteral (PN) nutrition prompts a lesser level of endogenous insulin secretion than EN nutrition (Muscelli *et al.* 2008), and thus may limit the incidence of hypoglycaemia due to unmodeled but enhanced insulin secretion resulting from

incretin effects. Plasma insulin responses to glucose given by gastric or jejunal intubation were significantly greater than those seen after IV infusion of the same glucose load in some studies (McIntyre *et al.* 1965). Likewise, Petrov and Zagainov (2007) reported a higher prevalence of hyperglycaemia during parenteral than enteral nutrition. Hence, if the risk of hyperglycaemia could be mitigated via advanced modelling methods, the potential benefits of the incretin effect could aid patient recovery. In addition, enteral nutrition is also associated with a significantly lower incidence of infection, sepsis and bacterial translocation that may reduce the need for surgical interventions to control pancreatitis and a reduced length of hospital stay (Marik and Raghavan 2004).

5.4.2 GLP-1 and GIP hormones

GLP-1 and GIP are both incretin hormones regulating postprandial insulin secretion. It is reported that both incretin hormones released from the gut, are essential in maintaining normoglycaemia and glucose homeostasis (Nauck *et al.* 1986; Schirra *et al.* 1996; Vilsbøll and Holst 2004). Conventionally, the incretin effect is evaluated by comparing the insulin and C-peptide concentrations to isoglycaemic oral and intravenous glucose tests (Nauck *et al.* 1986) which the increased insulin and C-peptide response during oral feeding defined as degree of the incretin effect.

Some studies found that postprandial GIP concentrations are higher than GLP-1 concentrations. However, others found GIP and GLP-1 are equally potent with respect to insulin secretion (Scrocchi *et al.* 1996), whereas some finding clarified GLP-1 to be three to five times more potent than GIP (Nauck *et al.* 1993; Toft-Nielsen *et al.* 1998). Previous studies also have investigated that GIP does not affect insulin secretion at fasting plasma glucose concentrations (Nauck *et al.* 1993; Nauck *et al.* 1997). Conversely, GLP-1 concentration in the fasting state and during an oral glucose challenge is considerably lower (Nauck *et al.* 1993). Thus, the contributions of GIP and GLP-1 to the incretin effects even in normal physiological condition with small changes in glucose concentrations remain unclear.

Modeling GLP-1/GIP with respect to insulin secretion and β -cell glucose sensitivity could potentially validate all the assumptions in evaluating the incretin effects. This proposed approach

will validate the finding presented here, as it will allow simultaneous estimation of both β -cell glucose sensitivity and the ability of GLP-1 and GIP to enhance insulin secretion. Hence, this study provides motivation and estimate of effect size and confounders to design such a study.

5.5 Summary

The findings of this analysis show the distinct existence of an incretin effect as an observable aspect of critically ill patient physiology. The findings were consistent with the presence of an EN-related incretin effect in a majority of critically ill patients. Clinically, the existence of this effect at EN nutrition transitions should also be considered in the management of glycaemia and could influence design of this therapy. Finally, while the results observed valid surrogates of the incretin effects, a prospective study with direct measurement and powered by these results may be required to confirm the outcomes directly.

Chapter 6. Endogenous Insulin Secretion Control Model

This chapter investigates the relationship between endogenous insulin secretion and changes in glucose concentrations using a proportional-derivative (PD) control model. The proposed control model is designed to investigate the endogenous insulin secretion amongst subjects with different metabolic states: normal glucose tolerance (NGT), impaired glucose tolerance (IGT) and type 2 diabetes mellitus (T2DM). The underlying effects that influence insulin secretion i.e. incretin effects are also defined by tracking the control model gain/response and the identified insulin sensitivity (S_I).

6.1 Introduction

Glucose homeostasis is primarily achieved by a balance between β -cell secretory function and insulin dynamics in cells and tissues. The two most common groups associated with the metabolic syndrome are IGT and T2DM that are characterised by IR and/or defects of β -cell function (DeFronzo *et al.* 1979). Thus, numerous tests have been developed to assess glucose induced insulin secretion and glucose-insulin pharmacodynamics/kinetics.

The close and inverse relationship between insulin secretion and insulin sensitivity has been widely acknowledged only during recent years (Bergman *et al.* 2002; Cobelli *et al.* 2007; Docherty *et al.* 2009; Toffolo *et al.* 1999). An attempt at finding a mathematical relationship between insulin sensitivity and pancreatic sensitivity to glucose was described by Bergman *et al.* (1981). Although, the application of modelling to clinical research has been slow, the intravenous (IV) glucose with minimal model simplified models has been the most frequently used approach (Breda *et al.* 2001; Pacini *et al.* 1998; Toffolo *et al.* 2001).

It is known that oral glucose stimulates insulin secretion over and above intravenous glucose (McIntyre *et al.* 1965; Perley and Kipnis 1967), and that the gastrointestinal hormones play an important role. These hormones (incretins) known as glucagon-like peptide (GLP-1) and glucose dependent insulintropic polypeptide (GIP), which enhanced the endogenous insulin secretion (Nauck *et al.* 1986; Vilsbøll and Holst 2004). GLP-1 and GIP are released from β -cells of the

islets of Langerhans in the pancreas following oral glucose consumption or meal, and each has shown to potentiate glucose-dependent insulin secretion. The insulinaemic GLP-1 response is blunted and the β -cell response to GIP is impaired in diabetic individuals (Muscelli *et al.* 2008; Nauck *et al.* 1986; Nauck *et al.* 1993; Toft-Nielsen *et al.* 1998). Hence, impaired incretin effects contribute to the β -cell incompetence of diabetes (Nauck *et al.* 1986). A study proved that introducing GLP-1 hormone can normalise blood glucose of diabetic individuals by stimulating glucose-induced insulin secretion, which has strengthened the incretin theory (Creutzfeldt and Ebert 1985; Deacon 2012; Hermansen *et al.* 2002).

Quantitative indices of insulin secretion can also be defined by plasma C-peptide concentrations in response to changes in glucose concentration which elucidate insulin sensitivity (Cretti *et al.* 2001; Mari *et al.* 2002). As C-peptide is equimolarly released with insulin but not degraded in the liver (Rubenstein *et al.* 1969) it is possible to evaluate insulin secretion via C-peptide models (Eaton *et al.* 1980; Van Cauter *et al.* 1992). Thus, exploiting particular mathematical models of insulin kinetics, glucose-insulin dynamics and C-peptide kinetics models would provide direct measurement of pancreatic β -cell secretion and hepatic insulin extraction (Mari *et al.* 2002; Pacini and Mari 2003; Polonsky *et al.* 1986; Watanabe *et al.* 1989).

Thus, a PD feedback-control model is used to precisely estimate the insulin secretion as a function of the changes in glucose concentration when plasma C-peptide data unaccounted. Consequently, the proposed control model would stimulate the insulin secretion while the PD control gains would segregate the possible causes of the metabolic dysfunction and its effects (i.e. incretin effects and/or *IR*).

6.2 Physiological models

6.2.1 Dynamics Insulin Sensitivity and Secretion Test (DISST) Model

The Dynamic Insulin Sensitivity and Secretion Test (DISST) provides quantitative measures of both S_I and U_N (Lotz *et al.* 2010; McAuley *et al.* 2011; McAuley *et al.* 2007). The dynamic DISST test is similar to the insulin modified IVGTT which uses an alternative modelling approach and a higher intensity test. The DISST S_I value is highly correlated to the EIC ($R=0.81$) (Lotz 2007; McAuley *et al.* 2011), and the test can contrast insulin secretion characteristics across patient groups with different levels of IR (McAuley *et al.* 2011). The DISST model consists:

$$\dot{C} = -(k_1 + k_3)C + k_2Y + \frac{U_N}{v_p} \quad 6.1$$

$$\dot{Y} = -k_2Y + k_1C \quad 6.2$$

$$\dot{I} = -n_kI - n_L \frac{I}{1+\alpha_I I} - \frac{n_I}{v_p}(I - Q) + \frac{U_{ex}}{v_p} + (1 - x_L) \frac{U_N}{v_p} \quad 6.3$$

$$\dot{Q} = -\left(n_C + \frac{n_I}{v_q}\right)Q + \frac{n_I}{v_q}I \quad 6.4$$

$$\dot{G} = -p_g(G - G_b) - S_I(GQ - G_bQ_b) + \frac{P_t}{v_g} \quad 6.5$$

Table 6.1-6.2 indicate the nomenclatures that define DISST model, *a-priori* exogenous input and the population parameters used in DISST model.

Table 6.1 Nomenclatures of DISST model.

| Variable | Value | Unit | Description |
|----------|------------|----------------------|--------------------------------------|
| C | stimulated | mU.L^{-1} | Plasma C-peptide concentration |
| Y | stimulated | mU.L^{-1} | Interstitial C-peptide concentration |
| U_N | stimulated | mU.min^{-1} | Endogenous insulin secretion rate |
| I | stimulated | mU.L^{-1} | Plasma insulin concentration |
| Q | stimulated | mU.L^{-1} | Interstitial insulin concentration |
| G | stimulated | mmol.L^{-1} | Blood glucose concentration |

Table 6.2 The exogenous input and the population parameters used in DISST model.

| Variable | Value | Unit | Description |
|-----------------|-----------------|------------------------|--|
| G_b | <i>a-priori</i> | mmol.L ⁻¹ | Basal blood glucose concentration |
| Q_b | <i>a-priori</i> | mU.L ⁻¹ | Basal interstitial insulin concentration |
| k_1, k_2, k_3 | <i>a-priori</i> | min ⁻¹ | C-peptide transport rates |
| V_g | <i>a-priori</i> | L | Glucose distribution volume |
| V_p | <i>a-priori</i> | L | Plasma insulin distribution volume |
| V_q | <i>a-priori</i> | L | Interstitial insulin distribution volume |
| n_K | <i>a-priori</i> | min ⁻¹ | Renal insulin clearance rate |
| n_L | <i>a-priori</i> | min ⁻¹ | Hepatic insulin clearance rate |
| x_L | <i>a-priori</i> | 1 | Fractional first-pass hepatic insulin extraction |
| n_I | <i>a-priori</i> | min ⁻¹ | Plasma-interstitial diffusion rate |
| n_C | <i>a-priori</i> | min ⁻¹ | Interstitial insulin degradation rate |
| U_{ex} | <i>a-priori</i> | mU.min ⁻¹ | Exogenous insulin input rate |
| P_t | stimulated | mmol.min ⁻¹ | Exogenous glucose input rate |
| p_g | 0.004 | min ⁻¹ | Non-insulin mediated glucose disposal rate |
| α_I | 0.0017 | L.mU ⁻¹ | Hepatic insulin clearance saturation parameter |

6.2.2 Stomach and gut model

The additional stomach and gut model is defined by Equation 6.6 to 6.8. The proposed model is simplified from a system model of oral glucose absorption (Lin *et al.* 2011). The steady exogenous glucose input rate (P_t) in Equation 6.5 is replaced with the term in Equation 6.8. P is the glucose appearance rate in bloodstream after oral glucose ingestion in stomach and gut. It is only applied in OGTT responses.

$$\dot{P}_1 = -d_1 P_1 + D \quad 6.6$$

$$\dot{P}_2 = -d_2 P_2 + d_1 P_1 \quad 6.7$$

$$P = \min(d_2 P_2, P_{max}) \quad 6.8$$

The nomenclature defining the two compartment stomach and gut are shown in Table 6.3 with constant population parameters.

Table 6.3 Nomenclature of stomach and gut model.

| Variable | Value | Unit | Description |
|-----------|------------|------------------------|--|
| P_1 | stimulated | mmol | Glucose appearance in stomach |
| P_2 | stimulated | mmol | Glucose appearance in gut |
| P | stimulated | mmol.min ⁻¹ | Glucose appearance in bloodstream from oral meal |
| P_{max} | 6.11 | mmol.min ⁻¹ | Maximum glucose flux from gut to plasma |
| d_1 | 0.1 | min ⁻¹ | Glucose transport rate from stomach to gut |
| d_2 | 0.0347 | min ⁻¹ | Glucose transport rate from gut to plasma |
| D | 75 | g | Oral glucose load |

The parameter values (d_1 and d_2) are reported to be the maximum rate of glucose appearance in both compartments.

6.2.3 PD feedback-control model of U_{en}

To reach glucose homeostasis, the body uses closed-loop, feedback-control system that defines the rate of insulin secretion in response to glucose and insulin concentrations. The proportional-derivative (PD) controller defines participant-specific U_{en} profiles that are function of increasing glucose (derivative control, ϕ_D) and glucose above basal (proportional control, ϕ_P).

The PD feedback-control model of U_{en} used in estimating the insulin secretion characteristics is defined:

$$U_{en} = U_b + \phi_P(G - G_b)\mathbb{H}(G - G_b) + \phi_D\dot{G}\mathbb{H}(\dot{G}) \quad 6.9$$

where: U_{en} is a controlled model of insulin secretion (mU.min⁻¹); U_b is basal insulin (mU.min⁻¹); ϕ_P and ϕ_D are the proportional and derivative gains (mU.L.mmol⁻¹.min⁻¹) and (mU.L.mmol⁻¹); $\mathbb{H}(x)$ is a Heaviside function that renders the coefficients of ϕ_P and ϕ_D equal to zero if the argument (x) is negative.

6.3 Hypothesis

The U_{en} control model consists of proportional and derivative (PD) controller gains. The proportional controller gain (ϕ_P) exhibits the static and steady-state change of glucose concentrations while the derivative controller gain (ϕ_D) signifies the dynamic change of glucose concentrations over time. It is hypothesised that the dynamic of U_{en} response is dominated by ϕ_D as the magnitude of $\phi_D > \phi_P$. Thus, high resolution and precision of measured data are important in the U_{en} control model as ϕ_D is particularly sensitive to assay error, and thus may lead to indistinct controller gains.

The incretin effects may potentially be segregated by comparing U_{en} control model responses between OGTT (oral glucose consumption) and DISST (IV glucose bolus) cohorts. A study by Bergman *et al.* (2003) showed a 26% higher insulin requirement to maintain normoglycaemia for the same amount of food consumption in patients who consumed parenteral nutrition compared with those who had enteral feeding. Thus, the incretin effects may be elucidated by comparing $\frac{\phi_D}{\phi_P}$ across DISST and OGTT responses. It is assumed that incretin effects occurred when $\frac{\phi_D}{\phi_P}$ OGTT $> \frac{\phi_D}{\phi_P}$ DISST.

6.4 Subjects and Methods

6.4.1 Participants

Fifty participants with varying degrees of glucose tolerance were recruited as part of the DISST validation trial (McAuley *et al.* 2011). These participants also underwent the euglycaemic clamp (EIC) (DeFronzo *et al.* 1979), the 4-sample oral glucose tolerance test (OGTT) and DISST within 8 days with at least one day between tests (Lotz 2007). Tests were given in random order based on their order of recruitment such that each of the six possible combinations was equally represented. The main outcome of this prior study by Lotz (2007) was to obtain insulin sensitivity metrics derived from the DISST, EIC and OGTT data.

On the morning of their first, basic metrics in calculating their BMI (weight, height, age, gender) was recorded. They were also required to complete a brief questionnaire concerning family history of type 2 diabetes mellitus and personal medical history.

These 50 participants were passively recruited via flyers and newspaper advertisements from Christchurch region of New Zealand in accordance with the conditions of the approved ethical consent. The participants were categorised in six subgroups to ensure the study cohort represented wider population. The six subgroups were defined as five lean males (BMI<25), five lean females, ten overweight males (BMI>25, BMI<30), ten overweight females, ten obese males (BMI>30), and ten obese females.

For simplicity, three subgroups were re-constructed and defined:

1. 10 lean participants (BMI<25); [5 males, 5 females]
2. 20 overweight participants (BMI>25, BMI<30); [10 males, 10 females]
3. 20 obese participants (BMI>30); [10 males, 10 females]

The cohort details were summarised and presented in Table 6.4 and Table 6.5.

Table 6.4 Participant details summary from the OGTT/DISST validation study. They were categorised in three groups (Lean, Overweight and Obese).

| Lean participant | Gender (F/M) | Age (yrs) | Weight (kg) | BMI (kg.m⁻²) | Fasting glucose (mmol.L⁻¹) | Fasting insulin (mU.L⁻¹) | NGT IGT* T2DM* |
|-------------------------------|---------------------|------------------|--------------------|--------------------------------|--|--|---------------------------|
| Q ₁ | 5 F | 25 | 59 | 20 | 4.1 | 2.6 | 10 NGT |
| Q ₂ | 5 M | 28 | 63 | 22 | 4.3 | 3.8 | |
| Q ₃ | | 30 | 72 | 23 | 4.4 | 5.2 | |
| Overweight Participant | Gender (F/M) | Age (yrs) | Weight (kg) | BMI (kg.m⁻²) | Fasting glucose (mmol.L⁻¹) | Fasting insulin (mU.L⁻¹) | NGT IGT* T2DM* |
| Q ₁ | 10 F | 29 | 70 | 26 | 4.2 | 4.8 | 18 NGT 2 IGT |
| Q ₂ | 10 M | 43 | 84 | 28 | 4.5 | 7.4 | |
| Q ₃ | | 50 | 87 | 29 | 4.8 | 9.6 | |
| Obese Participant | Gender (F/M) | Age (yrs) | Weight (kg) | BMI (kg.m⁻²) | Fasting glucose (mmol.L⁻¹) | Fasting insulin (mU.L⁻¹) | NGT IGT* T2DM* |
| Q ₁ | 10 F | 37 | 89 | 32 | 4.5 | 8.0 | 17 NGT 2 IGT 1 T2DM |
| Q ₂ | 10 M | 44 | 106 | 35 | 4.8 | 10.4 | |
| Q ₃ | | 53 | 118 | 39 | 5.0 | 22.7 | |

*based on 2hr OGTT glucose criteria of the ADA (2006).

Table 6.5 Overall cohort characteristics from OGTT/DISST validation study.

| Overall Participant | Gender (F/M) | Age (yrs) | Weight (kg) | BMI (kg.m⁻²) | Fasting glucose (mmol.L⁻¹) | Fasting insulin (mU.L⁻¹) | NGT IGT* T2DM* |
|----------------------------|---------------------|------------------|--------------------|--------------------------------|--|--|---------------------------|
| Q ₁ | 25 F | 29 | 72 | 26 | 4.3 | 4.6 | 45 NGT 4 IGT 1 T2DM |
| Q ₂ | 25 M | 41 | 85 | 29 | 4.6 | 8.0 | |
| Q ₃ | | 50 | 99 | 33 | 4.8 | 11.2 | |

*based on 2hr OGTT glucose criteria of the ADA (2006).

Due to inconsistency and assay error in two participants (1 NGT, 1 IGT) during OGTT assessment, only 48 participants were considered in this analysis.

6.4.2 Insulin sensitivity tests

6.4.2.1 DISST protocol

Participants had a cannula inserted into the antecubital fossa (large vein in the inner elbow) for blood sampling and bolus administration. A three-way tap was connected to the cannula to facilitate the extraction of 'dead-space' during sampling and enable flushing of boluses. Blood samples were drawn at $t=0$, 10, 15, 25 and 35 minutes and glucose, insulin and C-peptide concentrations were measured on these samples. A 10g bolus glucose (50% dextrose) was given at $t=5$ minutes and 1U of Actrapid insulin was administered immediately after the $t=15$ minutes sample. Participants were required to remain at the clinic for 30 minutes after the test and were provided with a small meal or snack. Due to the occasional difficulty in maintaining a free flowing cannula, some blood samples were not taken precisely at the time defined by the protocol. The consistency of the samples measurement in a dynamic test is critical to the accuracy of the resultant metrics. Thus, a timer device was used to record the actual times of the samples and boluses administered to the participants.

A purpose built sample timing program was installed on a palm-held computer with a purpose-built software package installed. The program displayed a large countdown timer for each scheduled sample. The trained clinician recorded the actual times (up to 1-second resolution) that the samples and boluses were performed by pressing a single button. The software was encoded in visual basic studio (.NET).

6.4.2.2 OGTT protocol

Participants recruited in this study had an OGTT for assessment diabetes status. Participants were given a standard lightly carbonated 75g glucose drink, immediately after a fasting blood sample. A cannula was placed in the antecubital fossa to enable blood samples. Further blood samples were collected at $t=30$, 60 and 120 minutes. Glucose and insulin concentrations were

measured in each sample. All samples were assayed for glucose at the bedside, spun and frozen for later insulin assays.

Current diabetes diagnosis criteria from ADA (2006b) includes either 120-minute glucose assay greater than 11.1 mmol.L^{-1} , fasting glucose $>7.0 \text{ mmol.L}^{-1}$ (126 mg.dL^{-1}) or/and HbA1c (glycated haemoglobin, which identifies average plasma glucose concentration) $>7\%$ ($53 \text{ mmols.mol}^{-1}$) can be used to diagnose diabetes. This must be confirmed on a second test on another day with 120-minute glucose values from the OGTT are between $G \geq 6.1$ and $G < 7.0 \text{ mmol.L}^{-1}$ (≥ 110 and $< 126 \text{ mg.dL}^{-1}$), which indicates impaired glucose tolerance.

6.4.2.3 Assay techniques

Glucose assays were analyzed using YSI 2300 stat plus Glucose and L-Lactate analyzer using whole blood. These were converted to plasma glucose, using Equation 6.9. Samples for insulin and C-peptide were immediately separated. Then the samples were frozen. Measurements of insulin were undertaken by the Endolab, Canterbury Health Laboratories using Roche Elecsys® after PEG precipitation of immunoglobulins (Roche Diagnostics, Mannheim, Germany). All C-peptide measurements were also undertaken by Endolab, Canterbury Health Laboratories using the Roche Elecsys® method. Serum cholesterol and triglycerides were measured enzymatically with Roche kits and HDL was measured in the supernatant after precipitation of apolipoprotein B containing lipoproteins with phosphotungstate/magnesium chloride solution (Assmann *et al.* 1983).

$$G_{plasma} = \frac{G_{wholebloodglucose}}{(2.4 \times 10^{-3}) \times haematocrit(\%)} \quad 6.10$$

6.4.3 Parameter Identification

The analysis was conducted with the DISST methodology defined in study by McAuley *et al.* (2011). Initially, the prior study results was used to define participant-specific U_b , n_L , x_L , S_L , and V_g parameter values as well as glucose and U_{en} profiles for DISST response using experimental data (McAuley *et al.* 2011) with Equation 6.1 to 6.5 and iterative integral method (IIM) (Docherty *et al.* 2012).

The a-priori parameter identification of participant-specific U_b , n_L , x_L , S_I , and V_g

The C-peptide model was de-convoluted using linear interpolation of the plasma C-peptide data (C_{interp}) (Lotz *et al.* 2010). Equation 6.2 can thus be solved analytically for $Y(t)$.

$$Y_t = k_1 \int_0^t C_{interp} e^{-k_2(t-\tau)} d\tau \quad 6.2a$$

This term was incorporated into the integral formulation of Equation 6.1:

$$C_{t1} - C_{t0} = k_1 k_2 \int_{t0}^{t1} \int_0^\tau C_{interp} e^{-k_2(t-\tau)} d\tau dt - (k_1 + k_3) \int_{t0}^{t1} C dt + \int_{t0}^{t1} \frac{U_N}{Vp} dt \quad 6.1a$$

Equation 6.1a was rearranged for the participant's endogenous insulin secretion response to the test stimulus, U_N .

$$\frac{U_N(t_1) - U_N(t_0)}{V_p} = C_{t1} - C_{t0} - k_1 k_2 \int_{t0}^{t1} \int_0^\tau C_{interp} e^{-k_2(t-\tau)} d\tau dt + (k_1 + k_3) \int_{t0}^{t1} C dt \quad 6.1b$$

Thus, U_N was defined using Equation 6.1b at a 1-minute resolution between $t=0$ and $t=end$.

The participant's insulin concentration response to the test stimulus was defined using the physiological model of Equation 6.3 and 6.4, the *a-priori* parameter values for each participant were discussed in detail in DISST validation study (Lotz 2007; McAuley *et al.* 2011) using Van Cauter's mathematical formula (Van Cauter *et al.* 1992) and U_N profile identified with Equation 6.1b.

To estimate minute-wise insulin profiles, insulin data was linearly interpolated between $t=0$ and end at the end of the test. The corresponding interstitial insulin concentration (Q) was identified using the analytical solution of Equation 6.4 with assumption of $Q_0 = 0.5I_0$ (Pretty 2012).

$$Q_i = e^{-\int_0^i n_c + \frac{n_I}{V_q}} (Q_0 + \frac{n_I}{V_q} \int_{t_0}^i e^{\int_0^i n_c + \frac{n_I}{V_q}} I dt) \quad 6.4a$$

where i = the test sample time.

Thus, profiles for both plasma and interstitial insulin responses to the test stimulus were defined. The IIM (Docherty *et al.* 2012) was used to identify participant-specific hepatic clearance (n_L) and extraction (x_L) values. The integral formulation of Equation 6.3 was separated into the coefficients of the known and unknown parameters:

$$n_L \underbrace{\int_{t_0}^{t_1} \frac{I}{1 + \alpha_I I} dt}_{CN} + x_L \underbrace{\int_{t_0}^{t_1} -U_N dt}_{CX} = \underbrace{I_{t_1} - I_{t_0} - \int_{t_0}^{t_1} [n_K I - \frac{n_I}{V_p} (I - Q) + \frac{U_{ex}}{V_p}] dt}_C \quad 6.3a$$

These coefficients were identified corresponds to the sample times of the test that began from $t_0=0$ to $t=t_1, t_2, \dots, t_{end}$. This approach allowed the generation of a matrix equation in terms of n_L and x_L to be formed:

$$\begin{bmatrix} CN_{t_0}^{t_1} & CX_{t_0}^{t_1} \\ CN_{t_0}^{t_2} & CX_{t_0}^{t_2} \\ CN_{t_0}^{t_3} & CX_{t_0}^{t_3} \\ \vdots & \vdots \\ CN_{t_0}^{t_{end}} & CX_{t_0}^{t_{end}} \end{bmatrix} \begin{bmatrix} n_L \\ x_L \end{bmatrix} = \begin{bmatrix} C_{t_0}^{t_1} \\ C_{t_0}^{t_2} \\ C_{t_0}^{t_3} \\ \vdots \\ C_{t_0}^{t_{end}} \end{bmatrix} \quad 6.3b$$

Thus, n_L and x_L were able to be constrained as necessary and identified using linear least square methods. The plasma and interstitial insulin concentration profiles were re-simulated using the DISST physiological model (Equations 6.3 and 6.4) with the identified hepatic clearance and extraction parameters.

The physiological stimulation of Q was then used with the glucose data and the IIM (Docherty *et al.* 2012) to identify participant-specific values for S_I and V_g . A linear interpolation was then used as an estimate of the glucose response to the test stimulus. The integral formulation of Equation 6.5 was rearranged and separated into the coefficients of the known and unknown parameters. The coefficients of the glucose model parameters were evaluated over the sample

times from $t_0=0$ to $t=t_1, t_2, \dots, t_{\text{end}}$, at the end of the test. These periods were chosen to minimise the fitting error and the variability of the identified insulin sensitivity (Docherty *et al.* 2011). Thus, a second matrix formulation was defined in terms of S_I and V_g .

$$S_I \underbrace{\int_{t_0}^{t_1} (GQ - G_b Q_b) dt}_{CS} + \frac{1}{V_g} \underbrace{\int_{t_0}^{t_1} P_t dt}_{CV_g} = \underbrace{G_{t_1} - G_{t_0} - \int_{t_0}^{t_1} [p_G I - \frac{n_I}{V_p} (I - Q) + \frac{U_{ex}}{V_p}] dt}_C \quad 6.5a$$

A matrix formulation was then defined in terms of S_I and V_g in Equation 6.5b:

$$\begin{bmatrix} CS_{t_0}^{t_1} & CV_g^{t_1} \\ CS_{t_0}^{t_2} & CV_g^{t_2} \\ CS_{t_0}^{t_3} & CV_g^{t_3} \\ \vdots & \vdots \\ CS_{t_0}^{t_{\text{end}}} & CV_g^{t_{\text{end}}} \end{bmatrix} \begin{bmatrix} S_I \\ V_g \end{bmatrix} = \begin{bmatrix} C_{t_0}^{t_1} \\ C_{t_0}^{t_2} \\ C_{t_0}^{t_3} \\ \vdots \\ C_{t_0}^{t_{\text{end}}} \end{bmatrix} \quad 6.5b$$

Bounds were placed on the value of V_g to reduce the effect that incomplete mixing might have on the sensitivity term. The values of V_g are limited to within 12 to 25% of the participant's bodyweight, per published data (Lotz 2007; Lotz *et al.* 2010; McAuley *et al.* 2011). Evaluation of Equation 6.5b yielded participant-specific S_I and V_g .

The same methodology with participant-specific (U_b , n_L , x_L and V_G) defined in prior study (McAuley *et al.* 2011) used to identify S_I and P for OGTT response. P is determined using Equation 6.8 from stomach and gut model.

The ϕ_P and ϕ_D controller gains identification

Then, Equation 6.3 and 6.9 were combined to individually identify ϕ_P and ϕ_D in Equation 6.11. The Equation 6.11 was then separated into the known (X) and unknown parameters (ϕ_P and ϕ_D).

$$\begin{aligned} \dot{I} = & \underbrace{-n_k I - n_L \frac{I}{1 + \alpha_I I} - \frac{n_I}{V_p} (I - Q) + \frac{U_{ex}}{V_p} + \frac{(1 - x_L)}{V_p} U_b + \dots}_{\text{known}(X)} \\ & \underbrace{\dots + \frac{(1 - x_L)}{V_p} [\phi_P (G - G_B) \mathbb{H}(G - G_B) + \phi_D \dot{G} \mathbb{H}(\dot{G})]}_{\text{unknown } (\phi_P \text{ and } \phi_D)} \end{aligned} \quad 6.11$$

The physiological stimulation of Q obtained in Equation 6.4a was then applied to Equation 6.11 using the *a-priori* parameters (U_b , n_L , x_L , S_I , and V_g) of DISST and measured insulin data from DISST and OGTT ($U_{ex} = 0$) tests. The known parameters and data are gathered, that can be defined as X in Equation 6.11a:

$$X = -n_k I - n_L \frac{I}{1 + \alpha_I I} - \frac{n_I}{V_p} (I - Q) + \frac{U_{ex}}{V_p} + \frac{(1 - x_L)}{V_p} U_b \quad 6.11a$$

Lastly, Equations 6.11(a-e) was then used with insulin and glucose data from DISST and OGTT tests to identify ϕ_P and ϕ_D using IIM, which generates the linear least square equations (similar to n_L and x_L parameter identification process in Equation 6.3a and 6.3b):

$$\dot{I} = X + \phi_P \frac{(1 - x_L)}{V_p} (G - G_B) \mathbb{H}(G - G_B) + \phi_D \frac{(1 - x_L)}{V_p} \dot{G} \mathbb{H}(\dot{G}) \quad 6.11b$$

$$\dot{I} - X = \phi_P \frac{(1 - x_L)}{V_p} (G - G_B) \mathbb{H}(G - G_B) + \phi_D \frac{(1 - x_L)}{V_p} \dot{G} \mathbb{H}(\dot{G}) \quad 6.11c$$

$$\underbrace{\phi_P \frac{(1-x_L)}{V_p} \int_0^i (G - G_B) \mathbb{H}(G - G_B) dt}_{C\phi_P} + \underbrace{\phi_D \frac{(1-x_L)}{V_p} \int_0^i \dot{G} \mathbb{H}(\dot{G}) dt}_{C\phi_D} = \underbrace{I_i - I_0 - \int_0^i X dt}_{RHS} \quad 6.11d$$

$$\begin{bmatrix} C\phi_P^{t_1} & C\phi_D^{t_1} \\ C\phi_P^{t_2} & C\phi_D^{t_2} \\ \vdots & \vdots \\ C\phi_P^{t_{end}} & C\phi_D^{t_{end}} \\ RR * GG & -GG \end{bmatrix} \begin{bmatrix} \phi_P \\ \phi_D \end{bmatrix} = \begin{bmatrix} RHS^{t_1} \\ RHS^{t_2} \\ \vdots \\ RHS^{t_{end}} \\ 0 \end{bmatrix} \quad 6.11e$$

where: the coefficients of the ϕ_P and ϕ_D model parameters were evaluated over the sample times from $t_0=0$ to $t=t_1, t_2, \dots, t_{end}$, at the end of the test. RR implies a ratio between ϕ_P and ϕ_D and GG

defines the magnitude of the penalty for deviation from the defined ratio (relative to the least squares fit).

The ϕ_P and ϕ_D values were identified in Equation 6.11(e) for each test response with $RR = 30$ implying that $\phi_D \rightarrow 30\phi_P$ and GG is 1 giving a relatively weak weighting to this ratio. Model residuals and interpretation of population trends were used to assess the performance of this PD control model.

The plasma and interstitial insulin concentration profiles were re-simulated using physiological model (Equation 6.3 and 6.4) with the identified ϕ_P and ϕ_D values for both DISST and OGTT assessments. Also, U_{en} control model response in Equation 6.9 was stimulated for comparison between NGT and IGT/T2DM.

6.4.4 Statistical analysis

The performance of this U_{en} (PD) control model was assessed via model residuals and interpretation of population trends between the DISST and OGTT model. Insulin model residuals (ϕ_I) are defined with Equation 6.12

$$\phi_I = \frac{1}{n} \sum (I_{measured}(t) - I_{fitted}(t)) \quad 6.12$$

where blood samples were collected at $t=t_0, t_1, t_2, \dots, t_n$ for the measurement of plasma glucose and insulin respectively. The mean residual for G was computed similarly.

The Kolmogorov-Smirnov (K-S test) is sensitive to differences in both location and shape of the empirical cumulative distribution functions of the two distributions. Thus, the K-S test was used to distinguish difference between DISST and OGTT parameter in distributions. All statistical analysis computed in this chapter using MATLAB (R2011b) (MathWork, Inc., Natick).

6.5 Results and discussion

The responses of the U_{en} control model were used to investigate and compare the dynamics of the metabolic effects for DISST and OGTT responses. Both controller gains of ϕ_P and ϕ_D

engaged with glucose concentrations to accurately stimulate the true insulin secretion after glucose ingestion.

6.5.1 Controller gains (ϕ_P) and (ϕ_D)

The relationship between U_{en} control model response and glucose concentration are defined as the sum of two controller gains. ϕ_P represents the relationship between insulin secretion and glucose concentration over steady state concentration. ϕ_D represents the dependence of insulin secretion on the rate of change in glucose concentration. This rate sensitivity effectively anticipates glucose concentrations rise over time.

Linear correlations in Figure 6.1(A) are observed between controller gains of ϕ_P and ϕ_D clarified that the PD control model is well-functioned in estimating U_{en} concentration. The control model summarised the linear correlation between controller gains and U_{en} control model response. Figure 6.1(A) shows that the DISST's ϕ_D and ϕ_P has correlation of $R=1$ and $R=0.96$ for OGTT. Additionally, Figure 6.1(B) shows ϕ_D of the OGTT response is correlated by $R=0.42$ with ϕ_D of DISST response while Figure 6.1(C) indicates that ϕ_P of OGTT response is correlated by $R=0.49$ with ϕ_P of DISST response.

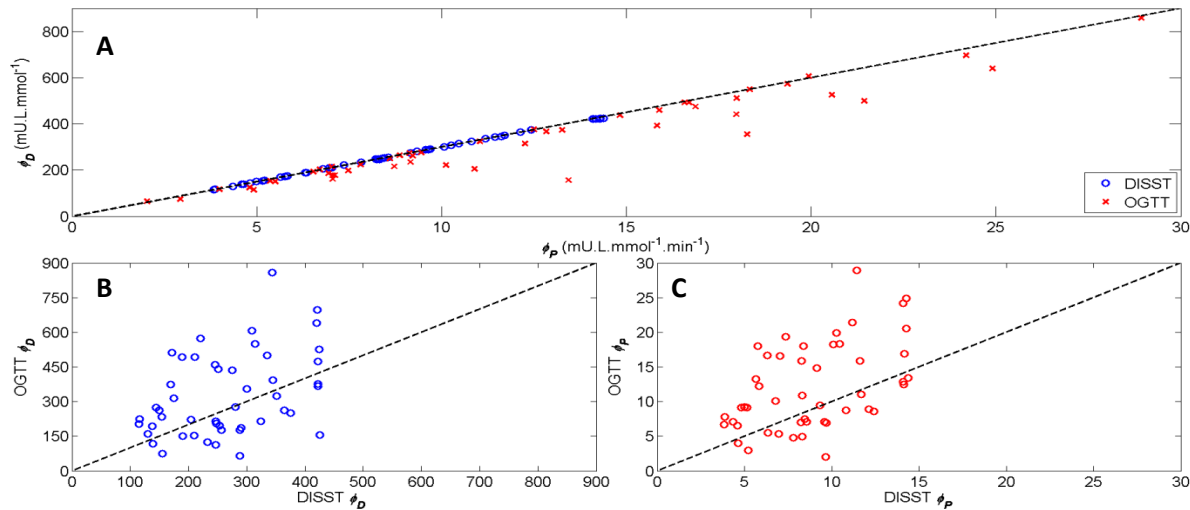


Figure 6.1 (A) Distribution of ϕ_D and ϕ_P for all participants underwent DISST and OGTT. (B) ϕ_D (C) ϕ_P controller gains comparison between OGTT and DISST responses.

The identified controller gains of ϕ_D and ϕ_P for both responses (DISST and OGTT) are shown in Table 6.6 and Figure 6.1. Figure 6.1(B and C) shows that ϕ_D and ϕ_P controller gains for the

OGTT response are only marginally greater than the DISST response. The median ϕ_D and ϕ_P of OGTT population response is increased by 10% and 20% compared to DISST, respectively, as in Table 6.6. The magnitude of ϕ_D are higher than ϕ_P due to the ratio constraint. ϕ_D portrays the magnitude of insulin secretion (β -cell function) with the changes of glucose fluxes. After oral/IV ingestion, increased glucose fluxes cause an increased rate of endogenous insulin secretion. While the ϕ_P controller gain only traces the absolute glucose concentration above the basal (minimum) level. Thus, ϕ_D defines the dominant behaviour in producing insulin in response to the glucose fluxes after glucose ingestion. Overall, results clearly show that the U_{en} control model response is led predominantly by ϕ_D controller gain as this gain parameter signifies the sensitivity of the model control due to changes in glucose concentration over time.

Table 6.6 Identified controller gains using IIM for both DISST and OGTT responses.

| Identified parameters using IIM | Median [IQR] | | <i>p</i> -value |
|---|---------------------------|---------------------------|-----------------|
| | DISST | OGTT | |
| ϕ_D (mU.L.mmol ⁻¹) | 252.36 [181.90,339.53] | 268.80 [189.42,467.03] | 0.014 |
| ϕ_P (mU.L.mmol ⁻¹ .min ⁻¹) | 8.43 [6.07,11.32] | 10.50 [7.06,16.78] | 0.003 |
| $\frac{\phi_D}{\phi_P}$ (min) | 29.96 [29.87,30.04] | 28.4 [25.2,29.5] | <0.0001 |

The most critical parameter in influencing observation of incretin effect is the proportional ratio between ϕ_D and ϕ_P controller gains. However, it was found that the median $\frac{\phi_D}{\phi_P}$ of OGTT and DISST responses were approximately the same (Table 6.6). This indicates that incretin effects were unobservable during oral glucose ingestion. Due to limited low resolution data of glucose and unmeasurable of GLP-1 and/or GIP concentrations in the OGTT assays it is suggested that the incretin effects can hardly be observed as the DISST model unable to capture the dynamics (via model fits) of this metabolic effects. OGTT consists only four glucose data points over 120-minute assessment which is insufficient to segregate the incretin effects as U_{en} control model response integrated with more complicated dynamics to modulate the true physiological U_{en} as glucose concentration changes over time. Furthermore, the OGTT response involves another dynamic input to the PK-PD models as oral glucose ingestion requires stomach and gut pathway for glucose appearance in bloodstream. The variable rate of glucose appearance during the

OGTT is difficult to model and requires further glucose measurements through gut. Thus, high resolution measurements of glucose concentrations and C-peptide measurements are essential to investigate the complexity of incretin effects in OGTT response.

An additional stomach and gut model assumption was introduced to define the unknown glucose appearance rate in OGTT response. Consequently, the glucose-insulin PD model stimulated slow and steady-state rate of glucose appearance (P) after glucose absorption in gut (P_2) and the rate of glucose appearance in blood (d_2) and S_I were identified using IIM. This stomach and gut model assumption was simplified from a nutrition model proposed by Lin *et al.* (2011). Model parameter were initially set to $d_1=0.1$ (min^{-1}), $d_2=0.0347$ (min^{-1}) (Lin *et al.* 2011) before the parameter identification process of S_I . The decay of glucose appearance rate is set to be constant 0.0347 min^{-1} (the half-life of glucose appearance in bloodstream is assumed to be 20 minutes). Thus, the U_{en} response amongst OGTT tests will either over or under-estimate the ‘real’ insulin secretion due to difficulty in estimating individual P and d_2 values which linked to the measured glucose.

The distribution of $\frac{\phi_D}{\phi_P}$ OGTT and $\frac{\phi_D}{\phi_P}$ DISST responses as a function of S_I is illustrated in Figure 6.2. The $\frac{\phi_D}{\phi_P}$ DISST responses are tightly bound to 30 as the model assumption implies that $\phi_D \rightarrow 30\phi_P$ despite the modest gain applied to the model. Thus, the U_{en} responses of DISST reflect the true physiological insulin secretion due to the changes in glucose concentrations. However, the $\frac{\phi_D}{\phi_P}$ OGTT responses are scattered due to the ‘unmeasured’ dynamics of the model assumption (i.e glucose appearance) after oral glucose ingestion. Presumably, the performance of U_{en} control model assumption in OGTT response is generally lower compared to DISST. OGTT assessment requires high resolution glucose data to perform the U_{en} control model in PK-PD model to capture the incretin effects after oral glucose ingestion.

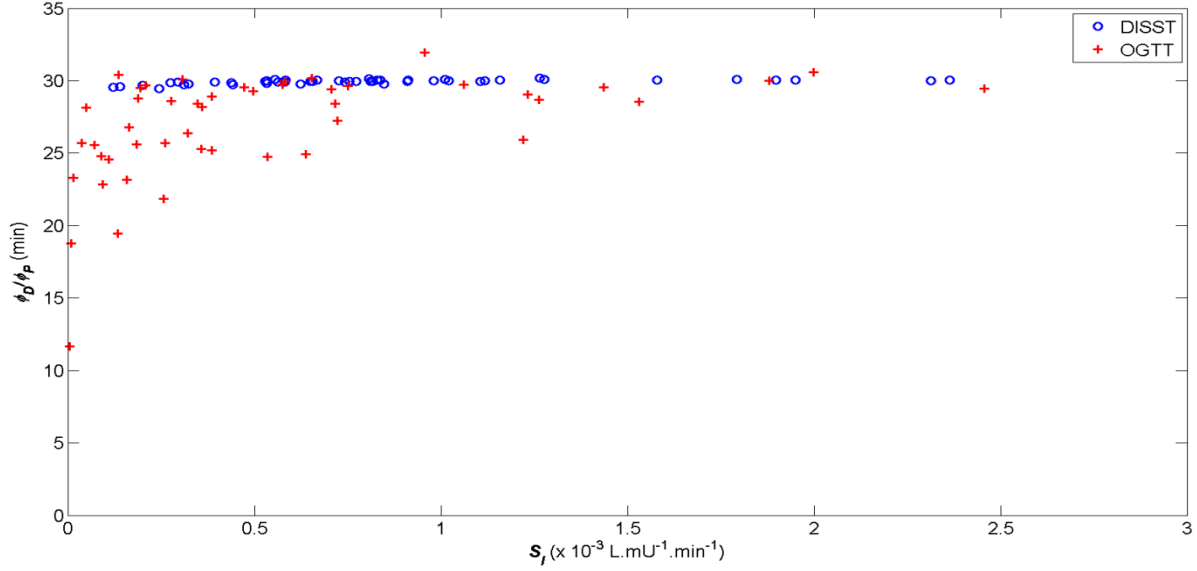


Figure 6.2 Distribution of $\frac{\phi_D}{\phi_P}$ OGTT and $\frac{\phi_D}{\phi_P}$ DISST responses as a function of S_I .

The modeled S_I is directly correlated to the response of interstitial insulin and glucose concentrations. In DISST and OGTT studies, there were four subjects diagnosed with IGT/T2DM based on the OGTT (ADA, 2006). *IR* is defined as abnormalities of S_I or insulin responsiveness (Kolterman *et al.* 1981). In particular, *IR* participants of the OGTT relied more heavily on the second phase insulin secretory response to glucose. This is supported by the literatures (Fehse *et al.* 2005; Garvey *et al.* 1985; Luzi and DeFronzo 1989).

6.5.2 Evaluation on U_{en} control model

The proposed U_{en} control model is designed to capture the relationship between U_{en} concentrations and controller gains of $(\phi_D$ and $\phi_P)$ which define the physiology of pancreatic β -cell glucose sensitivity (i.e. insulin secretion in response to changes in glucose concentrations). Due to the added model assumption of gut and stomach model in Equations 6.6–6.7 the glucose-insulin PD model has a slow rate of glucose appearance in blood after glucose passed through the gut and stomach compartments. This affects the modeled S_I . Therefore, U_{en} control model exhibited slow and steady U_{en} response amongst OGTT cohort compared to DISST cohort. The U_{en} response amongst DISST subjects was abrupt dynamic in counter-regulation the administration of IV glucose infusion at $t=5$ minutes after test commenced. Thus, the robustness

of the U_{en} control model was verified within these two different tests and the control model performance was reliable despite the limitation of measured data.

By specifically modelling U_{en} response of NGT, IGT and T2DM participants would be segregated the difference in insulin secretion referring to the participant's IR levels. Figure 6.3 illustrated the difference in U_{en} control model response of DISST and OGTT tests with three different IR levels.

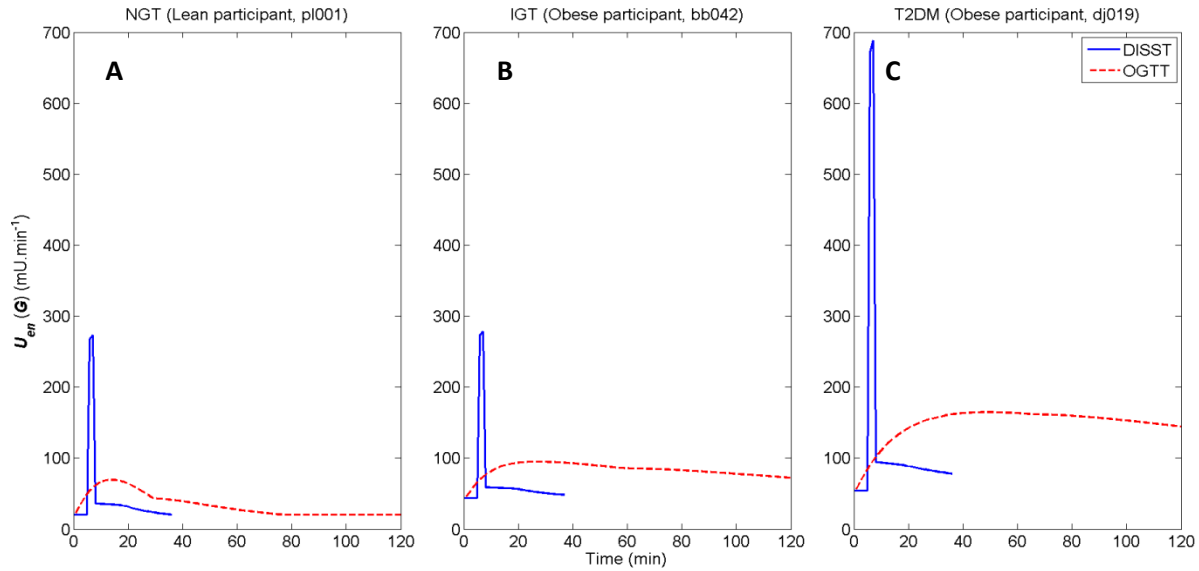


Figure 6.3 Difference of modeled U_{en} between NGT (A) lean, IGT (B) obese and T2DM (C) obese participants underwent DISST and OGTT.

The U_{en} responses and modeled S_I that correspond to controller gains (ϕ_D and ϕ_P) were evaluated for comparison using the same individuals by the OGTT and DISST assessments. It was found that the participant who had diabetes (T2DM) in Figure 6.3(C) had a higher insulin secretion than those with normal glucose tolerance (Figure 6.3(A)). In Figure 6.3(C), U_{en} response was greatly increased due to the reduced S_I of the T2DM participant. IR is the main contributor of increased demand on U_{en} secretion which causes hyperinsulinemia. Additionally, high insulin secretion in the obese participants with IGT/T2DM is also caused by failure in β -cell function to settle to basal insulin secretion after glucose ingestion.

U_{en} response time to the maximum estimated U_{en} rate amongst the OGTT cohort is variable between NGT and IGT/T2DM. The $U_{en(max)}$ response time for IGT/T2DM participants's are

approximately 30-50 minutes as in Figure 6.3(B and C). In contrast, the DISST average peak U_{en} response required only 7 minutes to reach the maximum estimated rate of insulin secretion. This explained the fast and quick action of U_{en} control model in reaching normoglycaemia state approximately 30-40 minutes after IV glucose consumption amongst DISST subjects. However, OGTT subjects attained longer period in reaching normoglycaemia (≈ 80 minutes) as the model control generated steady and slower U_{en} rate until it settled to baseline insulin secretion rate as in Figure 6.3(A).

The U_{en} control model responses of DISST with C-peptide data, DISST and OGTT were drawn for comparison in Table 6.7. Two U_{en} responses of first phase the total insulin secretion were calculated for each assay according to participant's IR states.

Table 6.7 Insulin secretion of first phase area under the curve, (U_I), and total area under the curve, (U_{Total}), of modeled U_{en} comparison between DISST with C-peptide, DISST and OGTT responses.

| Assay | | U_I (mU) Median [IQR] | U_{Total} (mU) Median [IQR] |
|------------------------------|--------------|----------------------------|----------------------------------|
| DISST with C-peptide data | NGT | 571 [465,739] | 2590 [1972,3391] |
| | IGT/T2DM | 609 [515,806] | 3668 [2686,4720] |
| | Total | 585 [465,739] | 2623 [2019,3412] |
| DISST | NGT | 936 [742,1234] | 2735 [2064,3569] |
| | IGT/T2DM | 1139 [841,1283] | 3621 [2751,4213] |
| | Total | 962 [742,1240] | 2813 [2120,3626] |
| OGTT | NGT | 2659 [1878,3746] | 7220 [5262,10107] |
| | IGT/T2DM | 3275 [2705,4791] | 15381 [11458,21213] |
| | Total | 2682 [1982,3746] | 8038 [5377,11735] |

The simplest method in assessing insulin secretion from OGTT data is the calculation of the measured insulin concentration area under the curve (AUC), over the whole test. AUC gives an idea of the amount of insulin that acts on the tissues, but lacks any information on the dynamics of the hormone in terms of secretion and clearance. To validate the U_{en} control model responses with the DISST study (with C-peptide data) by McAuley *et al.* (2011), Table 6.7 was constructed

to portray the estimated AUC between DISST and OGTT. The first phase (U_I) is defined by area under the curve at the first peak and the total secretion (U_{Total}) is the total area under the curve of insulin secretion. Both values were determined as population median [IQR]. Overall, the OGTT cohort delivered the highest U_I and U_{Total} . The DISST response has approximately the same amount of U_{Total} compared to DISST with C-peptide data. The DISST model with C-peptide data estimated U_{en} concentration directly from plasma C-peptide PK model to estimate U_{en} response.

6.5.3 Glucose and Insulin pharmacodynamics/kinetics model responses

DISST protocol was designed to improve upon the intravenous glucose tolerance test (IVGTT) protocol. The DISST protocol was comparatively low-dose insulin IV boluses and has shortened duration. Thus, to define insulin PK and glucose-insulin PD models of DISST protocol, the DISST model (Docherty *et al.* 2010; Lotz 2007) was designed with modification of the minimal model (Bergman *et al.* 1979) and the C-peptide PK model of Eaton *et al.* (1980).

Three participants model fit examples are shown in Figures 6.4-6.6 and verify that U_{en} control model captured different responses based on participant health (IR levels). The DISST validation study successfully identified participant-specific parameters (U_b , n_L , x_L , S_I , V_g) by modelling insulin PK and glucose-insulin PD models. This adaptation of this U_{en} control model in DISST model elucidates the relationship insulin secretion in response to changes in glucose concentration.

In general, Tables 6.8–6.10 show comparison between DISST and OGTT challenges (NGT, IGT and T2DM participants) of modeled S_I , $\frac{\phi_D}{\phi_P}$ controller gain response and total insulin secretion corresponds to the model response.

The model responses of 21 years old lean (BMI=19 kg.m⁻²), NGT participant are illustrated in Figure 6.4.

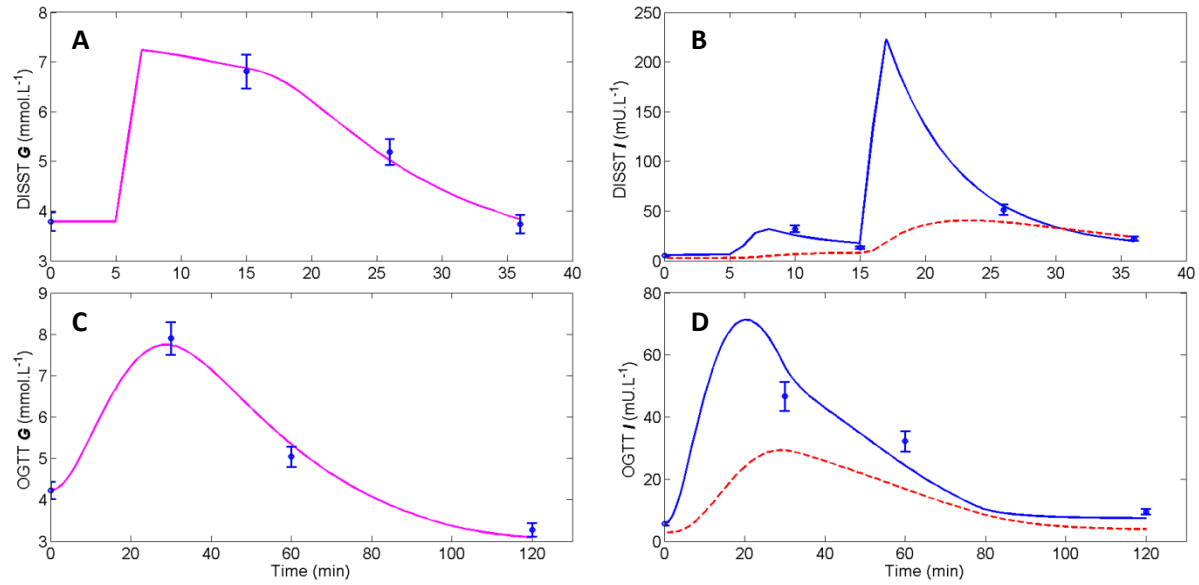


Figure 6.4 Model responses of a NGT participant underwent DISST; (A) glucose and (B) plasma and interstitial insulin, and OGTT; (C) glucose and (D) plasma and interstitial insulin.

Table 6.8 Comparison of identified parameters based on model responses of a NGT participant shown in Figure 6.4.

| NGT participant | S_I (L.mU ⁻¹ .min ⁻¹) | $\frac{\phi_D}{\phi_P}$ (min ⁻¹) | U_T (mU) |
|-----------------|---|--|---------------|
| DISST | 0.0010 | 30.04 | 1479 |
| OGTT | 0.0014 | 29.54 | 4019 |

It can be concluded that the PD control model was successfully applicable in DISST models particularly in this participant as good model fits with minimum residual are observed in Figure 6.4. Although modeled I and Q for this participant (Figure 6.4(A) and (B)) slightly drifted from measured I in the OGTT response, the model fit still delivered good residual with limited measured data. As $\frac{\phi_D}{\phi_P}$ controller gain response for the OGTT was equally the same as $\frac{\phi_D}{\phi_P}$ for the DISST, it may elucidate that the incretin effects is not significant when insulin is produced during glucose ingestion for the OGTT response. Thus, further investigation with high resolution

of glucose and C-peptide measurements is required to observe the incretin effects in OGTT responses.

Model responses of an IGT participant who underwent OGTT and DISST challenges are illustrated in Figure 6.5. This participant is borderline obese with a BMI \approx 30 kg.m⁻².

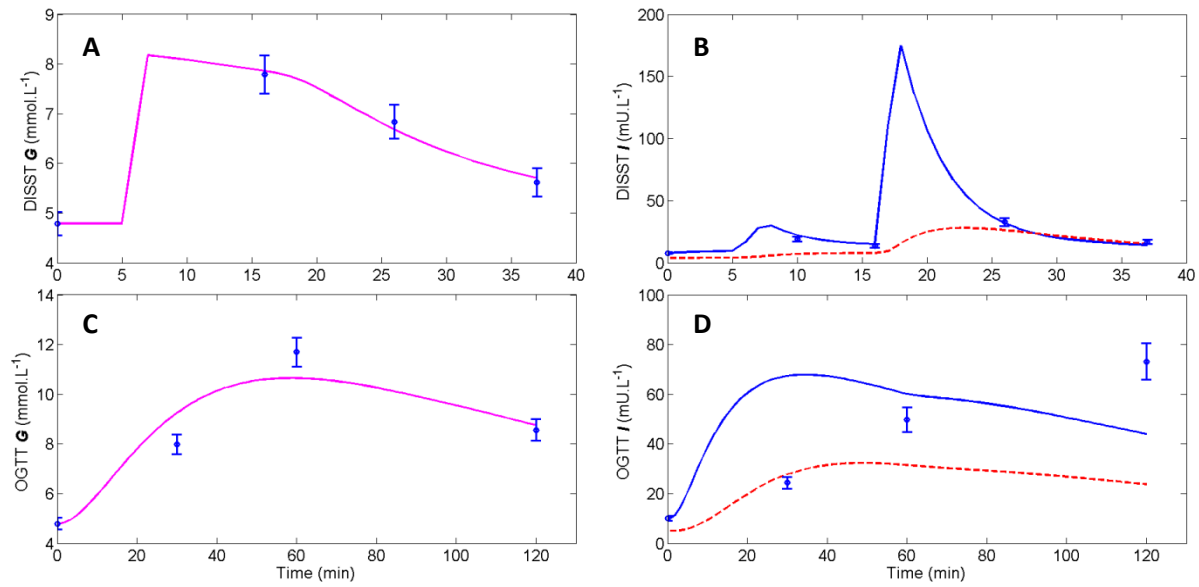


Figure 6.5 Model responses of an IGT participant underwent DISST; (A) glucose and (B) plasma and interstitial insulin, and OGTT; (C) glucose and (D) plasma and interstitial insulin.

Table 6.9 Comparison of identified parameters based on model responses of an IGT participant shown in Figure 6.5.

| IGT participant | S_I (L.mU ⁻¹ .min ⁻¹) | $\frac{\phi_D}{\phi_P}$ (min ⁻¹) | U_T (mU) |
|-----------------|---|--|---------------|
| DISST | 0.0008 | 29.93 | 3108 |
| OGTT | 0.0001 | 22.84 | 12921 |

The modeled G , I and Q model responses to the OGTT were poorly fitted. The variable rate of glucose appearance in bloodstream after glucose ingestion is difficult to model given only four available glucose measurements. Also, IR is compensated by increased insulin secretion (Larson & Ahren, 1998) which is illustrated in this IGT participant's U_{en} response. However, the total U_{en} responses of OGTT (Table 6.7 and 6.9) amongst IGT/T2DM participants were considerably over-estimated from true U_{en} obtained from DISST study with C-peptide data (McAuley *et al.*

2011). Thus, DISST model with OGTT response could not fully capture the dynamics and its effects within PK-PD models, thus reducing the parameter identifiability of S_I . Thus, the PD control model performance in OGTT responses is comparatively low via the proportional ratio of controller gains $\frac{\phi_D}{\phi_P}$ which causes high residual in model fit of I . However, this IGT participant delivered good model responses in term of controller gains $\frac{\phi_D}{\phi_P} \approx 30$ with DISST response as expected.

A T2DM participant's model responses underwent DISST and OGTT challenges are illustrated in Figure 6.6. This participant is obese with a BMI $\approx 42 \text{ kg.m}^{-2}$.

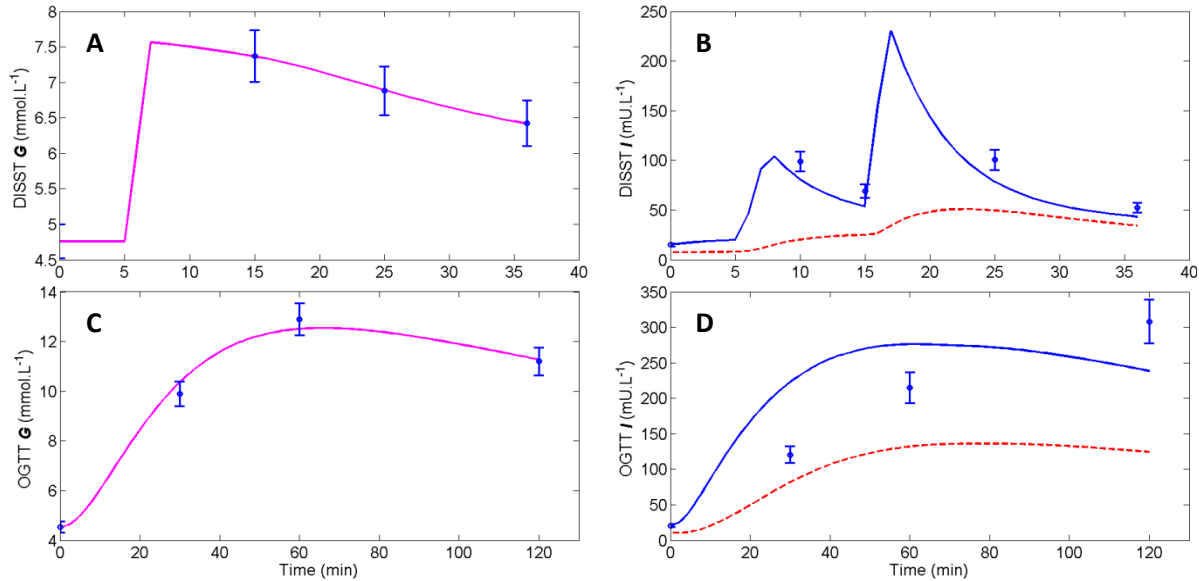


Figure 6.6 Model responses of a T2DM participant underwent DISST; (A) glucose and (B) plasma and interstitial insulin, and OGTT; (C) glucose and (D) plasma and interstitial insulin.

Table 6.10 Comparison of identified parameters based on model responses of a T2DM shown in Figure 6.6.

| T2DM participant | S_I ($\text{L.mU}^{-1}.\text{min}^{-1}$) | $\frac{\phi_D}{\phi_P}$ (min^{-1}) | U_T (mU) |
|------------------|---|---|---------------|
| DISST | 0.00013 | 29.54 | 4133 |
| OGTT | ≈ 0 | 11.64 | 17841 |

As a participant's IR level increases (especially during T2DM), the U_{en} model response is also increased. This is due to hyperinsulinaemia occurred in T2DM which excessive insulin secretion

apart from inability of glucose uptake by insulin binding receptor as target tissues fails to respond appropriately to insulin (Ferrannini *et al.* 1991; Weyer *et al.* 1999). This causes high glucose concentration and failure to settle to basal concentrations after oral/IV glucose. As a result, insulin sensitivity decreased. Again, results show that incretin effects are impossible to be observed in IGT/T2DM participants as $\frac{\phi_D}{\phi_P}$ of these participants is far more less than $\frac{\phi_D}{\phi_P}$ of the NGT participants. This incretin effect is highly suppressed and not significant due to other metabolic dysfunctions and the inability of U_{en} control model to capture this effect as low $\frac{\phi_D}{\phi_P}$ response is observed (Table 6.10). In this analysis small numbers (N=5) of participants associated with IGT/T2DM were identified. Therefore, further investigation with larger cohort of IGT/T2DM is required to investigate insulin secretion and incretin effects that affect this metabolic dysfunction using this approach.

6.5.4 Validation and residual

OGTT is not generally considered a surrogate assessment for S_I and is used specifically for the diagnosis of type 2 diabetes. It measures the body's ability to clear a 75-g glucose load, which indirectly computes insulin sensitivity based only on measured glucose concentrations with (if any) plasma insulin measurements. The OGTT protocol in this study only measured glucose and plasma insulin concentrations. Ideally additional measured C-peptide would further validate the participant-specific parameters from DISST validation study using pharmacokinetic model from Van Cauter's study (1992). Prior studies (Belfiore *et al.* 2001; Gutt *et al.* 2000; Matsuda and DeFronzo 1999; Soonthornpun *et al.* 2003; Stumvoll *et al.* 2000) proposed index of insulin sensitivity derived from OGTT with wide range of glucose tolerance and several have validated it against EIC ($R=0.56$, $p<0.001$) (McAuley *et al.* 2011).

Table 6.11 shows the population mean residual of G and I model responses for DISST and OGTT challenges. The model response in G for both challenges seemed consistent and accurate as the medians of population mean residual were calculated to be 0.06 mmol.L⁻¹ (DISST) and -0.16 mmol.L⁻¹ (OGTT). However, the median of population ϕ_I values was ~40 times the difference between the OGTT and DISST model responses.

Table 6.11 Population mean residuals (median, [IQR]) of G and I model responses for DISST and OGTT challenges.

| Mean residual | Median [IQR] | | p -value |
|-----------------------------|----------------------|------------------------|------------|
| | DISST | OGTT | |
| G (mmol.L ⁻¹) | 0.06 [0.02,0.12] | -0.16 [-0.57,-0.03] | <0.0001 |
| I (mU.L ⁻¹) | 0.05 [-0.45,0.43] | -2.19 [-6.08,0.48] | <0.0001 |

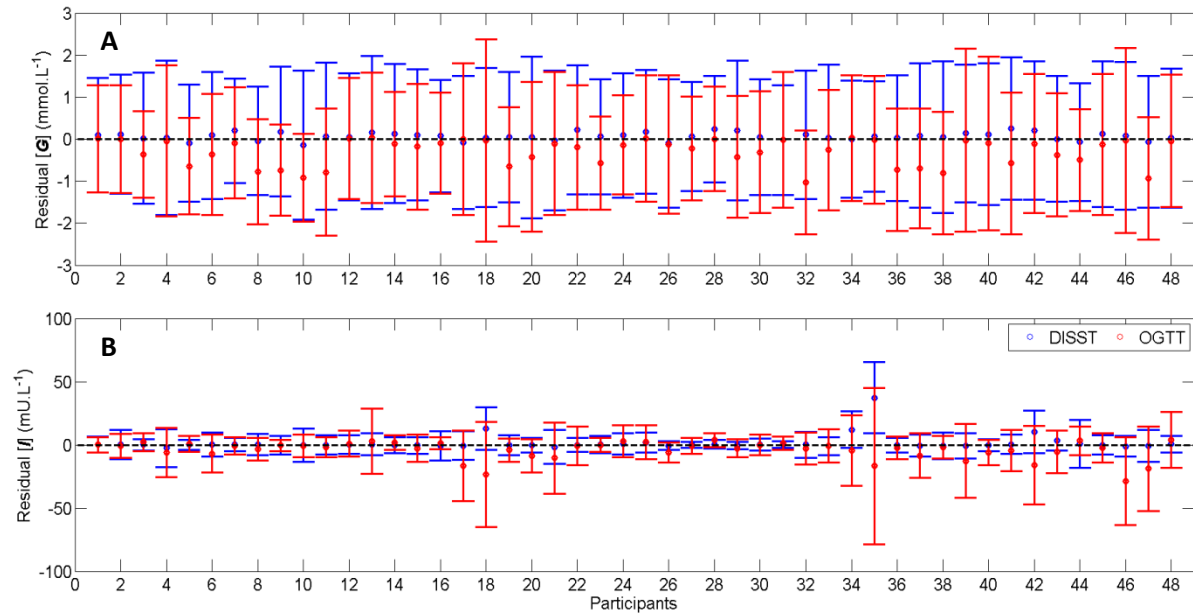


Figure 6.7 Mean residual per-participant between measured G (A) and I (B) data and model responses from DISST and OGTT.

The goodness plasma insulin and interstitial insulin model fit determines the performance of the U_{en} control model. Ambiguous glucose stimulus during the OGTT test causes poor model fits with high residual which overall generates variable U_{en} response. This high response variability might hinder the observation of underlying metabolic effects that enhance insulin secretion. The fitting error is potentially due to the low resolution of the OGTT glucose measurements. The model identifiability issues (Docherty *et al.* 2011) also limit the accuracy of ϕ_D controller gain to estimate the true U_{en} response.

Other factors which contribute to the high residual in I model response is the fairly poor estimation of U_{en} control model based only on the changes in glucose concentration and *a-priori*

parameter estimation of (n_L , x_L and U_b) from DISST study (McAuley *et al.* 2011). Due to limited data accessibility in identifying five parameters (n_L , x_L , ϕ_D , ϕ_P and U_b) in plasma insulin PK model, three out of these five parameters were assumed from the prior DISST study (McAuley *et al.* 2011). However, a study by Nauck *et al.* (1986) proved that after oral glucose ingestion, the estimated fractional hepatic insulin extraction, x_L , to be significantly lower than that during IV glucose infusion (63.4-76.5%). Thus, this factor slightly contributes to the overall discrepancy in I model response.

Overall, the U_{en} control model is generally well developed amongst normal participants, particularly in DISST response despite the limited measured data. The incretin effects were not significant in OGTT response in comparison to DISST after glucose ingestion. However, rough estimation of participant-specific parameters (n_L , x_L) and the imprecise estimation on endogenous insulin secretion baseline concentration (U_b) especially in IGT and T2DM participants in OGTT challenge might contribute to bias. Thus, a clear understanding of plasma insulin/interstitial PK models and glucose PD model is particularly essential in investigating the incretin effects and insulin-glucose interaction. Also, repeatability and variability study with additional measured C-peptide, GLP-1 and GIP hormones concentration in a large cohort which covering all levels of insulin resistance should be conducted to validate the proposed U_{en} control model.

6.6 Summary

The dynamic insulin secretion responses to changes in glucose concentration can be modeled using PD feedback-control approach. The new model allows the characterisation of both static (basal) and dynamic insulin secretion model responses which define the pancreatic β -cell glucose sensitivity parameters. The application of this model to various physiological states associated with alterations in insulin secretion and/or kinetics provides novel links into the role of plasma insulin/interstitial PK models and glucose-insulin PD model.

The potential to simultaneously assess β -cell glucose sensitivity during an OGTT adds significant value i.e. tracking and evaluating incretin effects amongst normal subject to the DISST model with additional gut and stomach model and U_{en} control model. This will require measurements of plasma C-peptide concentration and the use of the independent glucose-insulin

PD model and insulin/interstitial PK models (Docherty *et al.* 2010; Lotz 2007) for glucose-insulin regulatory mechanism. The proposed U_{en} control model of insulin secretion and kinetics will provide the ability to precisely define the pancreatic β -cell function in relation to insulin sensitivity.

Care must also be applied in adopting simplistic methods as assay errors can lead to compensations among parameters and consequently to inaccurate models of pancreatic β -cell function defined by ϕ_D and ϕ_P . The control model and methods proposed here which enable one to obtain a specific observation on both β -cell function and insulin kinetics in a single approach, delivered a good compromise between model simplicity and accuracy.

Chapter 7. Impact of Haemodialysis on Insulin Sensitivity and Insulin Kinetics

Glucose intolerance and hyperinsulinemia are common in acute renal failure patients (DeFronzo *et al.* 1978; Hartmann *et al.* 1997; Lebovitz 2001; Mak 2000; Nestler *et al.* 1988; Reaven 1988). Critically ill patients often have renal failure and are normally treated with haemodialysis therapy (Basi *et al.* 2005; Bellomo and Egi 2005). It is process that removes waste products such as urea and creatinine from the blood to replace kidney function (Hoste *et al.* 2003). Other dialysis therapies such as, Peritoneal Dialysis, Continuous Arteriovenous Hemofiltration (CAVH) or Continuous Arteriovenous Haemodialysis (CAVHD) are also used. However, these therapies are slower and require special procedures, which are intermittent or continuous, depending on patient condition (Bellomo and Egi 2005; Lauer *et al.* 1983). More importantly, the impact of haemodialysis treatment on the glycaemic behaviour of critically ill patients with ARF is still unclear. Given the strong links between glycaemic variability and clinical outcomes (Branco *et al.* 2005; Capes *et al.* 2000; Van den Berghe *et al.* 2001) it would be useful to know if this common therapy affects metabolic management. The study in this chapter investigates this question.

7.1 Introduction

Acute renal failure, (ARF) is a common complication among critically ill patients, especially for elderly patients with diabetes (Thomas *et al.* 2007). Approximately 36% of critically ill patients are diagnosed with ARF (De Mendonça *et al.* 2000; Metnitz *et al.* 2002; Uchino *et al.* 2005) with a significant proportion progressing to chronic renal failure requiring weekly haemodialysis, (HD), (Star 1998). Several epidemiological studies have shown an increase in morbidity and mortality following the development of ARF (Bentley 2011; Hoste *et al.* 2003; Mak 2000; Metnitz *et al.* 2002).

The increasing incidence of critically ill patients with ARF associated with increased insulin resistance may be explained by several factors, including a rising incidence of sepsis (Bagshaw *et al.* 2007; Marvin and Morton 2009), major surgery (especially cardiothoracic), nephrotoxic

medications, and chronic medical conditions (Hoste *et al.* 2003). With both uraemia and HD treatment, glycaemic control (GC) can be complicated (Mak 2000) as GC can affect insulin secretion, insulin clearance, gluconeogenesis (Van den Berghe *et al.* 2001) and peripheral tissue sensitivity of insulin (Shrishrimal *et al.* 2009). Importantly, HD treatment improves patient condition by removing waste and toxin from the blood. However, clinical studies have also shown that HD treatment clears plasma insulin through increased absorption (Abe *et al.* 2007; Kobayashi *et al.* 2000; Stenvinkel *et al.* 1992), which will affect glycaemic management and control. Overall, the effect of renal failure on metabolic kinetics in critically ill patients is unknown. These unknown effects have the potential to complicate metabolic management and treatment.

In particular, insulin resistance is persistent in many ARF patients (Mak 1995; Marvin and Morton 2009). Hence, these patients are at risk of developing hyperglycaemia (Mak 2000) with its associated negative outcomes (Krinsley 2003; Weekers *et al.* 2003). The mechanism of glucose intolerance in ARF patients is ambiguous (Hampers *et al.* 1966). DeFronzo *et al.* (1981) and Mak (1994) showed that insulin resistance among ARF patients improved during a 10 week course of HD treatment. However, the net effect of HD treatment on glycaemic regulation and insulin sensitivity (S_I) in a critically ill cohort is still unknown.

This analysis uses dense clinical data and a model-based analysis to investigate changes in a clinically validated, model-based S_I metric at HD transitions in a cohort of critically ill patients. We hypothesized that the observed S_I would decrease during HD due to enhanced insulin clearance compared to the model, and would be recaptured again when HD is stopped. These changes in modeled S_I would thus offer a unique observation on insulin kinetics and action in this population of critically ill patients with ARF that would better inform metabolic care.

7.2 Subjects and Methods

7.2.1 Patient cohort

Retrospective blood glucose (G) measurements, nutrition administration rates (P), and insulin delivery (U_{ex}) data for this study were obtained from the Specialized Relative Insulin Nutrition

Titration (SPRINT) study of 371 critically ill patients that required glycaemic control (Chase *et al.*, 2008). A total of 51 of 371 patients (14%) had acute renal failure (ARF) treated with HD. The exogenous insulin and nutrition given to these patients were optimized to maximise blood glucose time in the range between 4.0-7.0 mmol.L⁻¹, minimising hyperglycaemia, via patient-specific nutrition and insulin administration (Chase *et al.* 2008; Lonergan *et al.* 2006).

The 51 ARF patients were treated with haemodialysis (HD) with polysulfone (PS) dialyzer membrane (APS-15SA: Asahi Medical Co.,Ltd, Tokyo). This PS dialyzer membrane is reported to affect plasma insulin clearance during HD treatment (Abe *et al.* 2011; Jaber *et al.* 2000). Patients were haemodialysed three times weekly (in fasting state) for a minimum of 4 hours in the Christchurch Hospital Intensive Care Unit (ICU).

Study inclusion from the total of 51 ARF patients required a minimum of 5 hours of patient data before dialysis, followed by at least 6 hours of dialysis, and then at least 5 hours after dialysis. The clinical details of this cohort are summarized in Table 7.1. The APACHE III diagnosis for these patients can be divided into 5 main groups: Sepsis, Cardiovascular, Trauma, Respiratory and Diabetes. Full details on SPRINT can be obtained from Chase *et al.* (2008).

Table 7.1 SPRINT Cohort baseline variables (N=51). Data are expressed as median [IQR]. (APACHE = Acute Physiology and Chronic Health Evaluation).

| | Median | [IQR] |
|-----------------------------|---------------------------|--------------|
| Age (years) | 65 | [46-73] |
| % Male | 76% | |
| APACHE II score | 24 | [19-30] |
| APACHE III Diagnosis | Number of Patients | % |
| Trauma | 9 | 18 |
| Cardiovascular | 13 | 25 |
| Sepsis | 20 | 39 |
| Respiratory | 7 | 14 |
| *Diabetes | 2 | 4 |

*Patients diagnosed with Type 1 Diabetes Mellitus (T1DM)

7.2.2 Identification of S_I

Model-based S_I is identified hourly by fitting G measurements with estimated endogenous insulin secretion using the ICING-2 (Intensive Control Insulin-Nutrition-Glucose) model (Pretty 2012). An integral-based method (Hann *et al.* 2005) and clinical data are used to identify patient-specific stepwise S_I profiles with 1-hour resolution. The model nomenclature is in Equations 7.1–7.7 and is mathematically defined:

$$\dot{G} = -p_G G - S_I G \frac{Q}{1 + \alpha_G Q} + \frac{P + EGP - CNS}{V_G} \quad 7.1$$

$$\dot{Q} = n_I(I - Q) - n_C \frac{Q}{1 + \alpha_G Q} \quad 7.2$$

$$\dot{I} = -n_K I - n_L \frac{I}{1 + \alpha_I I} - n_I(I - Q) + \frac{U_{ex}}{V_I} + (1 - x_L) \frac{U_{en}(G)}{V_I} \quad 7.3$$

$$\dot{P}_1 = -d_1 P_1 + D \quad 7.4$$

$$\dot{P}_2 = -\min(d_2 P_2, P_{max}) + d_1 P_1 \quad 7.5$$

$$P = \min(d_2 P_2, P_{max}) + PN \quad 7.6$$

$$U_{en}(G) = \begin{cases} u_{min}, & u_{min} > k_1 G(t) + k_2 \\ k_1 G + k_2, & u_{min} \leq k_1 G(t) + k_2 \leq u_{max} \\ u_{max}, & u_{max} < k_1 G(t) + k_2 \end{cases} \quad 7.7$$

Tables 7.2–7.3 indicate the nomenclatures that define ICING-2 model, population parameters and kinetics values based on diabetic status and the exogenous input variables in ICING-2 model.

Table 7.2 Nomenclature of ICING-2 model.

| Parameters | Description | Unit |
|-------------|--------------------------------------|------------------------|
| G | Blood glucose level | mmol.L ⁻¹ |
| Q | Interstitial insulin level | mU.L ⁻¹ |
| I | Plasma insulin level | mU.L ⁻¹ |
| P_1 | Stomach glucose content | mmol |
| P_2 | Gut glucose content | mmol |
| P | Rate of glucose appearance in plasma | mmol.min ⁻¹ |
| $U_{en}(G)$ | Endogenous insulin secretion | mU.min ⁻¹ |

Table 7.3 Population parameters and kinetics values of ICING-2 model based on diabetic status.

| Parameters | Description | Value | Unit |
|------------|--|--------|------------------------------------|
| EGP | Endogenous glucose production | 1.16 | mmol.min^{-1} |
| CNS | Central nervous system glucose uptake | 0.3 | mmol.min^{-1} |
| p_G | Patient endogenous glucose removal | 0.006 | min^{-1} |
| S_I | Insulin sensitivity | | $\text{L.mU}^{-1}.\text{min}^{-1}$ |
| α_G | Saturation parameter of insulin-mediated glucose removal | 0.0154 | L.mU^{-1} |
| V_G | Plasma glucose distribution volume | 13.3 | L |
| n_I | Plasma-interstitium insulin diffusion rate | 0.006 | min^{-1} |
| n_C | Receptor-bound insulin degradation | 0.006 | min^{-1} |
| n_K | Renal insulin clearance | 0.0542 | min^{-1} |
| n_L | Hepatic insulin clearance | 0.1578 | min^{-1} |
| α_I | Saturation parameter for hepatic insulin clearance | 0.0017 | L.mU^{-1} |
| V_I | Insulin distribution volume | 4.0 | L |
| x_L | First pass hepatic clearance | 0.67 | 1 |
| d_1 | Rate of glucose transport through the enteral route into the bloodstream | 0.0347 | min^{-1} |
| d_2 | | 0.0069 | min^{-1} |
| P_{max} | Maximal gut glucose flux | 6.11 | mmol.min^{-1} |
| u_{min} | Minimum pancreatic secretion rate | 16.7 | mU.min^{-1} |
| u_{max} | Maximum pancreatic secretion rate | 266.7 | mU.min^{-1} |
| k_1 | Pancreatic insulin secretion glucose-sensitivity | *NGT: | 14.9 |
| | | *T2DM: | 4.9 |
| | | *T1DM: | 0.0 |
| k_2 | Pancreatic insulin secretion offset | *NGT: | -49.9 |
| | | *T2DM: | -27.4 |
| | | *T1DM: | 16.7 |
| U_{ex} | Intravenous insulin input rate | | mU.min^{-1} |
| D | Oral glucose input rate from enteral nutrition | | mmol.min^{-1} |
| PN | Intravenous glucose input rate from parenteral nutrition | | mmol.min^{-1} |

*Note: NGT=Normal Glucose Tolerance, T1DM=Type 1 Diabetes Mellitus, T2DM=Type 2 Diabetes Mellitus

Model estimation of endogenous insulin secretion $U_{en}(G)$ is in the range between 16.7mU.min^{-1} and 266.7mU.min^{-1} as a function of glycaemic concentration (G) (Pretty 2012). This overall metabolic model has been clinically validated with median prediction error less than 4-5% (Lin *et al.* 2011). The model has been used in several clinical glycaemic control trials and insulin sensitivity tests (Evans *et al.* 2011; Fisk *et al.* 2012; Pretty *et al.* 2012).

7.2.3 Hypothesis

The model uses fixed values for kidney clearance, n_K , and a G dependent model for endogenous insulin secretion, U_{en} . Therefore, the unmodeled changes due to HD or any other effect (Jamaludin *et al.* 2012) are reflected in modeled S_I . There are two dialysis transitions, OFF/ON and ON/OFF examined in this study.

OFF/ON transition

The HD PS membrane is known to absorb plasma insulin during dialysis treatment (Abe *et al.* 2011), which will lower plasma insulin levels compared to the model estimates. Therefore, it is hypothesised that after the OFF/ON transition, S_I will decrease given fixed model assumptions on U_{en} and.

ON/OFF transition

By using the same dialyzer membrane with fixed assumptions on U_{en} and n_K , it is hypothesise that S_I will increase as the plasma insulin levels recover to higher levels more similar to those modeled after HD treatment ends.

Overall, changes in S_I due to HD might be caused by either or both of:

- a) Changes in U_{en} due to HD treatment (Abe *et al.* 2011; Mak 1995).
- b) Changes in the effective insulin clearance (n_K in the model) (Abe *et al.* 2009; Zhang *et al.* 2008).

It is assumed that true S_I will remain (largely) constant over the short transition times for all patients. Thus, any larger changes in S_I consistent over the cohort and time around a transition may be described to the impact of HD therapy. However, tracking changes and the net effect on modeled S_I after both transitions cannot delineate the separate effects noted above. In particular, rising U_{en} with respect to fix model assumptions leads to an increase in observed S_I , while rising n_K leads to apparent reduction in S_I . If S_I is decreasing, it means the effect of insulin clearance increases outweigh the effect of U_{en} increases.

7.2.4 Data analysis

Numerical calculations and parameter identification were performed using MATLAB (The MathWorks Inc., Natick, MA). The proportional difference in S_I (ΔS_I) was calculated as:

$$\Delta S_I = 2 \frac{S_{I(t)} - S_{I(t-1)}}{S_{I(t)} + S_{I(t-1)}} \quad 7.8$$

Blood glucose changes, ΔG were calculated in a similar manner to assess any changes in glycaemia that could affect results.

This analysis uses a 2-hour moving average to reduce the effect of measurement error, and the influence of transient effects. S_I profiles are identified over periods starting 3 hours just before dialysis commencement until 4-10 hours after dialysis ends. This range ensures full settling of patient response after transitions. Patient-specific blood glucose and insulin sensitivity at both OFF/ON and ON/OFF dialysis transitions are illustrated distribution and Bland–Altman plots.

An analysis of p -values that cause ΔS_I was performed to investigate the impact of HD therapy on n_K and U_{en} . It is assumed that ΔS_I is constant and indistinct when $p=1$ is reached at certain n_K and U_{en} parameter values.

Non-parametric Wilcoxon rank-sum tests were used to assess ΔS_I and ΔG over the cohort at each transition. This test assesses two samples of independent observations that have distinct median values (Wilcoxon *et al.* 1970). Significant results ($p<0.05$) show a difference in median values for both independent observations.

7.3 Results and Discussion

This chapter investigated the impacts of HD treatment on insulin sensitivity, and insulin kinetics (based on renal insulin clearance, endogenous insulin secretion and the effective plasma insulin), through a clinically validated modeled ΔS_I metric at both OFF/ON and ON/OFF dialysis transitions.

7.3.1 Impacts of HD on S_I

Significant insulin sensitivity changes were observed at the OFF/ON dialysis transition ($p=0.02$) in Figure 7.1 and Table 7.4. This analysis indicates that modeled S_I decreased over the initial 4-hours after HD started and the changes occurred as rapidly as 2 hours. This result implies that HD significantly affected plasma insulin levels via changes in renal insulin clearance and/or endogenous insulin secretion, compared to baseline model assumptions.

Figure 7.1 shows ΔS_I over 6 hours at the OFF/ON and ON/OFF dialysis transitions. Patients diagnosed with pancreatitis, diabetes and other metabolic dysfunctions showed larger variance in ΔS_I ($>-150\%$). However, the trend at the OFF/ON transition in Figure 7.1(A) and the Bland-Altman plot of Figure 7.2 is much clearer. These results for both transitions are shown in detail in Table 7.4.

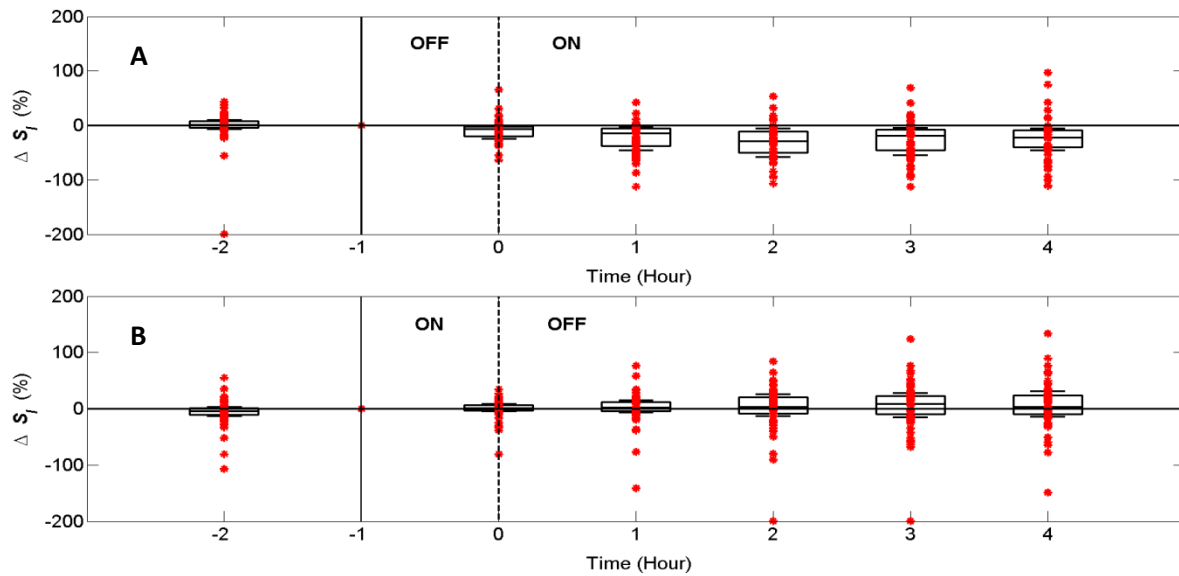


Figure 7.1 Summary of (2 hours before) and 4 hours after dialysis treatment commenced at OFF/ON (A) and ON/OFF (B) dialysis transition at $t=-2$ to $t=4$ hr (N=51).

Mak (2000) showed that ARF patients on HD treatment had lower S_I during constant hyperinsulinemia. Based on this finding, it is suggested that excessive insulin secretion amongst ARF patients with reduced insulin-mediated glucose uptake by tissues insulin resistance caused constant hyperinsulinemia. As a result, S_I decreases 2-3 hours after HD is commenced. However, tissue sensitivity to insulin is increased post-dialysis, as waste products (urea and creatinine)

were cleared from bloodstream (DeFronzo *et al.* 1978). Improvement in glucose tolerance is also observed and was accounted for by an increase in insulin secretion (48%) post-dialysis (DeFronzo *et al.* 1978). Thus, longer pre- and post-dialysis observations on ΔS_I would elucidate factors that influence insulin kinetics (insulin clearance or insulin secretion) in critically ill patients with ARF.

Table 7.4 summarises ΔS_I over the OFF/ON and ON/OFF transitions for a longer period to assess this issue. S_I decreased after the OFF/ON dialysis transition until $t=2$ hours, where it settled with median $\Delta S_I=-29\%$ (IQR: [-58, 6] %; $p<0.05$). There were a comparatively low number of confounders, indicating a relatively strong effect is observed. Median ΔS_I increased by a much more modest and not significant 9% for the ON/OFF transition (Table 7.4), (IQR: [-15, 28] %; $p=0.7$) at $t=3$ hours after the ON/OFF transition. The number of confounders is significantly higher for the ON/OFF transition and the p -values indicate that the hypothesized effect cannot be confirmed at this transition. G remains effectively constant at both transitions.

Table 7.4 Results at both OFF/ON and ON/OFF dialysis transitions of 6 hours with inverted S_I confounders ($t=-2$ to $t=4$ hr; $N=51$).

| Time t (hr) | OFF/ON (N=51), expect $\Delta S_I < 0$ | | | | | | ON/OFF (N=51), expect $\Delta S_I > 0$ | | | | | |
|------------------|--|--------------|--------------|------------|-----------------------------------|----|--|--------------|--------------|------------|-----------------------------------|----|
| | Q_1 (%) | Q_2 (%) | Q_3 (%) | p -value | $\Delta S_I > 0$ (confounders) | % | Q_1 (%) | Q_2 (%) | Q_3 (%) | p -value | $\Delta S_I < 0$ (confounders) | % |
| -2 | -7 | 1 | 10 | 0.9 | 31 | 61 | -13 | -4 | 3 | 0.7 | 32 | 63 |
| -1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 0 | -24 | -7 | -1 | 0.3 | 10 | 20 | -5 | 1 | 8 | 0.9 | 24 | 47 |
| 1 | -45 | -14 | -2 | 0.05 | 7 | 14 | -7 | 2 | 15 | 0.9 | 23 | 45 |
| 2 | -58 | -29 | -6 | 0.02 | 5 | 10 | -13 | 4 | 26 | 0.8 | 21 | 41 |
| 3 | -55 | -19 | -5 | 0.03 | 9 | 18 | -15 | 9 | 28 | 0.7 | 23 | 45 |
| 4 | -46 | -22 | -5 | 0.03 | 6 | 12 | -15 | 3 | 31 | 0.5 | 24 | 47 |

* p -values measured by using Wilcoxon rank sum tests, $Q_1=25\%$ percentile, $Q_2=50\%$ percentile, $Q_3=75\%$ percentile.

Abe *et al.* (2007) reported that when using glucose-free dialysate in diabetic patients, plasma glucose concentrations were significantly decreased from 2 hour after HD treatment commenced. As expected, plasma insulin concentrations were also significantly decreased besides glucose concentrations. Thus, S_I is decreasing immediately 2 hours after the OFF/ON transition, as shown in Figure 7.2(B). In addition, Figure 7.2(A) illustrated that ΔG decreases slightly once HD treatment started at $t=2$ h, showing some added clearance in that region.

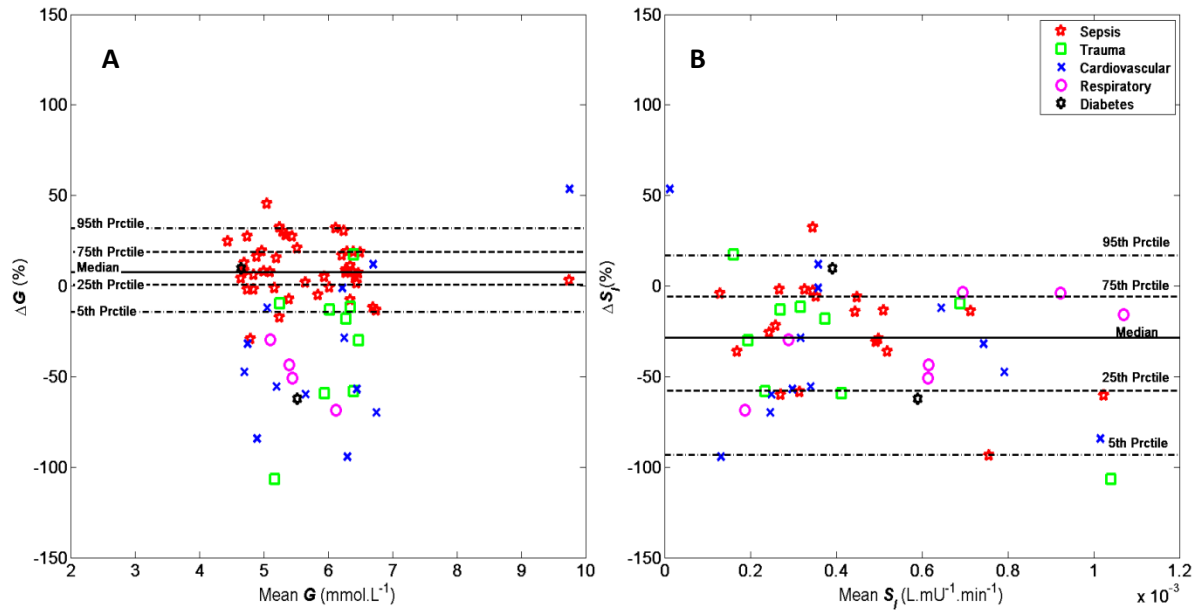


Figure 7.2 Bland-Altman ΔG (A) and ΔS_I (B) over the OFF/ON dialysis transition between $t=-1$ and 2 (N=51). Median $\Delta G=8\%$, Median $\Delta S_I=-29\%$.

A substantial change in S_I at the OFF/ON dialysis transition indicates a strong and fast process of cleaning and clearing toxic substances from blood leading to increased model-based S_I due to either decreased U_{en} or increased n_K clearance. However, at the ON/OFF dialysis transition, the reverse process is a lot slower physiologically. Hence, the model-based S_I , after HD in this study may be expected to remain largely unchanged, as observed here, even for extended periods after HD treatment (in Table 7.5).

An extended dialysis interval (>10 hours) of ΔS_I for both OFF/ON and ON/OFF dialysis transition across the N=26 subjects with sufficient data is shown in Table 7.5. S_I decreased during the OFF/ON dialysis interval until $t=8$ hours, where it settled to a median reduction of -25% (IQR: [-10, -51] %; $p=0.04$). There were only 2 confounders ($\Delta S_I > 0$) from 26 patients at

$t=8$ hours. However, while the ON/OFF transition results improved relative to the hypothesized effect, the results were still marginally insignificant ($p>0.07$), so that this effect is clearly smaller than hypothesized and cannot be effectively observed in this study.

Table 7.5 Extended results at both OFF/ON and ON/OFF dialysis transitions of >10 hours with inverted S_I confounders ($t=-2$ to $t=10$ hr; $N=26$).

| Time t (hr) | OFF/ON (N=26) | | | | | | ON/OFF (N=26) | | | | | |
|------------------|---------------|--------------|--------------|-------------|-----------------------------------|----------|---------------|--------------|--------------|------------|-----------------------------------|----|
| | Q_1 (%) | Q_2 (%) | Q_3 (%) | p -value | $\Delta S_I > 0$ (confounders) | % | Q_1 (%) | Q_2 (%) | Q_3 (%) | p -value | $\Delta S_I < 0$ (confounders) | % |
| -2 | -4 | 3 | 13 | 0.9 | 16 | 62 | -15 | -6 | 1 | 0.7 | 19 | 73 |
| -1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 |
| 0 | -25 | -10 | -2 | 0.4 | 4 | 15 | -5 | 3 | 9 | 0.8 | 10 | 28 |
| 1 | -44 | -23 | -4 | 0.1 | 3 | 12 | -7 | 8 | 17 | 0.8 | 11 | 42 |
| 2 | -56 | -30 | -6 | 0.09 | 2 | 8 | -8 | 13 | 32 | 0.6 | 9 | 35 |
| 3 | -55 | -19 | -2 | 0.09 | 6 | 23 | -4 | 18 | 42 | 0.3 | 9 | 35 |
| 4 | -53 | -24 | -4 | 0.1 | 4 | 15 | -13 | 14 | 36 | 0.3 | 10 | 28 |
| 5 | -53 | -29 | -8 | 0.08 | 4 | 15 | -12 | 22 | 44 | 0.2 | 10 | 28 |
| 6 | -44 | -25 | -8 | 0.1 | 4 | 15 | -6 | 23 | 47 | 0.1 | 8 | 31 |
| 7 | -40 | -21 | -9 | 0.07 | 3 | 12 | -5 | 18 | 50 | 0.07 | 7 | 27 |
| 8 | -51 | -25 | -10 | 0.04 | 2 | 8 | -5 | 19 | 39 | 0.07 | 7 | 27 |
| 9 | -47 | -18 | -7 | 0.04 | 1 | 4 | -6 | 22 | 38 | 0.07 | 8 | 31 |
| 10 | -45 | -12 | -3 | 0.09 | 5 | 19 | -4 | 22 | 41 | 0.08 | 8 | 31 |

* p -values measured by using Wilcoxon rank sum tests, $Q_1=25\%$ percentile, $Q_2=50\%$ percentile, $Q_3=75\%$ percentile.

Long-term (4.9 weeks) HD treatment has been shown to normalize insulin sensitivity and result in a marked improvement in glucose metabolism (Kobayashi *et al.* 2000), but this might not completely normalize glucose utilization (DeFronzo *et al.* 1981). It is impossible to delineate the

effects that contribute to changes in S_I in this study, due to model identifiability issues to separate S_I and U_{en} (Docherty *et al.* 2011). Over longer intervals, as in Table 7.5, inter-patient or intra-patient variation may further obscure the observation of the effect itself (Pretty *et al.* 2012).

Glucose intolerance among critically ill patients with ARF occurs with significant inhibition of insulin secretion and a state of peripheral insulin resistance (DeFronzo *et al.* 1978; Mak 1995) on top of insulin resistance from critical illness (Basi *et al.* 2005). It is also reported that in patients with ARF insulin resistance occurred even though glomerular filtration rate (GFR) values were still within normal range (Fliser *et al.* 1998). The effect of insulin resistance can be exacerbated by impairment in the role of insulin in maintaining the hepatic glucose balance (DeFronzo *et al.* 1981). Specifically, an inability of insulin to stimulate hepatic glucose uptake with decreasing S_I has been observed in ARF patients (Valera Mora *et al.* 2003). Thus, understanding the pharmacokinetics of insulin during dialysis is clinically important.

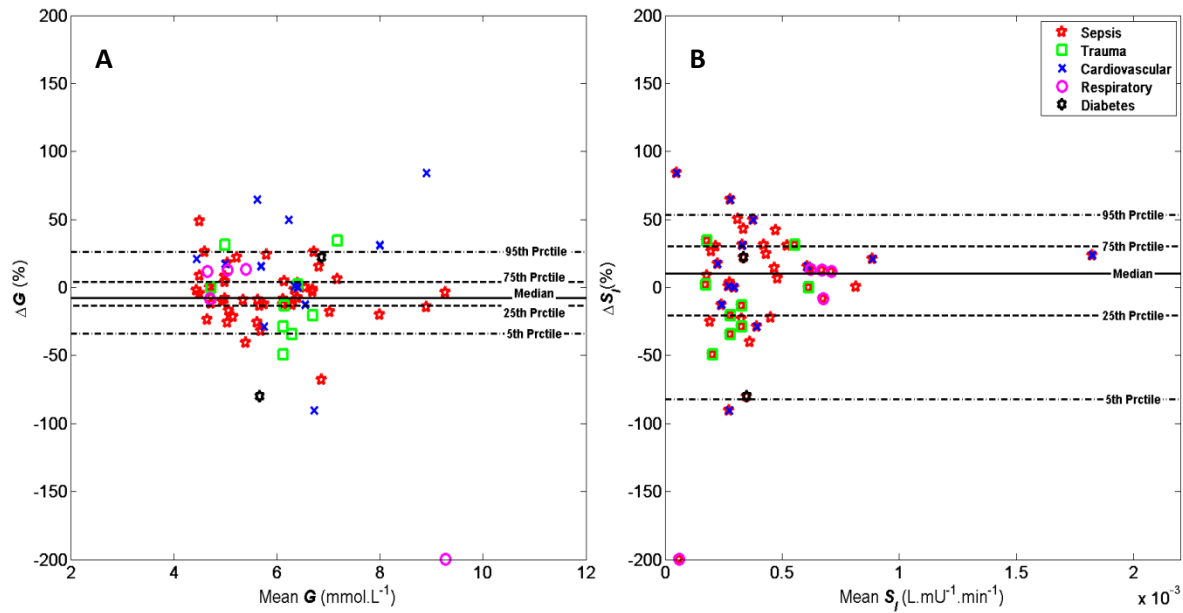


Figure 7.3 Bland-Altman of ΔG (A) and ΔS_I (B) over the ON/OFF dialysis transition between $t=-1$ and 2 (N=51). Median $\Delta G=-8\%$, Median $\Delta S_I=10\%$.

The modeled ΔS_I at the ON/OFF dialysis transition in this study was insignificant ($p>0.05$), as in Table 7.4-7.5 and in Figure 7.3. It is assumed that acute IV administration of 1,25-dihydroxyvitamin D₃ (1,25(OH)₂D₃) given to ARF patients during haemodialysis may increase insulin secretion and reverse glucose intolerance (Mak 1994). An improvement in glucose

metabolism has been observed in some studies via lower mean glucose during dialysis and more rapid disappearance rate of glucose in the immediate post-dialysis period (Alfrey *et al.* 1967). In general, glucose metabolism and renal function are expected to increase gradually after post-dialysis when toxic substances that are suspected of hindering renal function slowly rebuild their levels.

7.3.2 Impact of HD on n_K and U_{en}

Plasma insulin is reduced by enhanced insulin clearance due to the PS dialyzer membrane (Abe *et al.* 2011; Jaber *et al.* 2000) used during HD treatment in this study. It is suggested that the most significant reduction in plasma insulin during HD treatment is through absorption of insulin across the PS membrane (Abe *et al.* 2011), where the equilibrium amount of insulin absorbed was greatest in positively charged membranes (Zhang *et al.* 2008). A significant uptake and degradation of insulin may occur when renal insulin clearance significantly exceeds GFR (Mak 2000), as would occur in HD treatment. This enhanced insulin clearance rate and accumulation of dialyzable uraemic toxins inhibit insulin degradation. These waste products can be sufficiently cleared from bloodstream by HD treatment (Mak 1994).

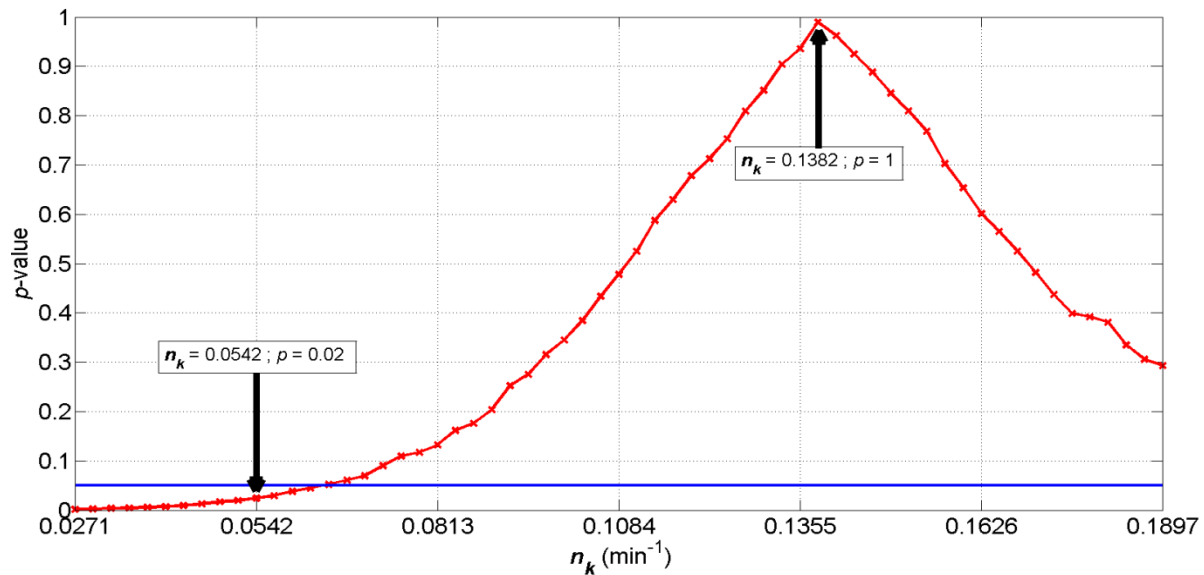


Figure 7.4 Renal insulin clearance rate corresponds to p -values that cause ΔS_I (\downarrow) of -29% at $t=2$ hours for the OFF/ON transition.

Figure 7.4 illustrates renal insulin clearance and corresponding to p -values with respect to variation in median ΔS_I at $t=2$ hours at OFF/ON transition. This analysis implies that n_K is

increased by 2.55-fold from the model assumed value of $0.0542 \text{ (min}^{-1}\text{)}$ after HD is commenced. Plasma insulin also is suspected to be absorbed through the membrane dialyzer and cleared from bloodstream during HD treatment on top of toxins and waste products (urea and creatinine). ΔS_I is not significant at the OFF/ON transition when $p > 0.05$. Thus, when p -value reaches 1 ($p=1$), it is assumed that the reduction of ΔS_I is remained constant and indistinct (-29%). Hence, increased modeled n_K supported the prior finding that plasma insulin might also be cleared during HD treatment via membrane dialyzer absorption or through the dialysate products.

It is suspected that U_{en} might also be affected by HD therapy. Thus, Figure 7.5 illustrates U_{en} and corresponding p -values that cause significant decreased ΔS_I of -29% at $t=2$ hours for the OFF/ON dialysis transition. Modeled endogenous insulin secretion rate with $p = 0.02$ clearly indicates that U_{en} also contributes to significant changes in S_I during HD.

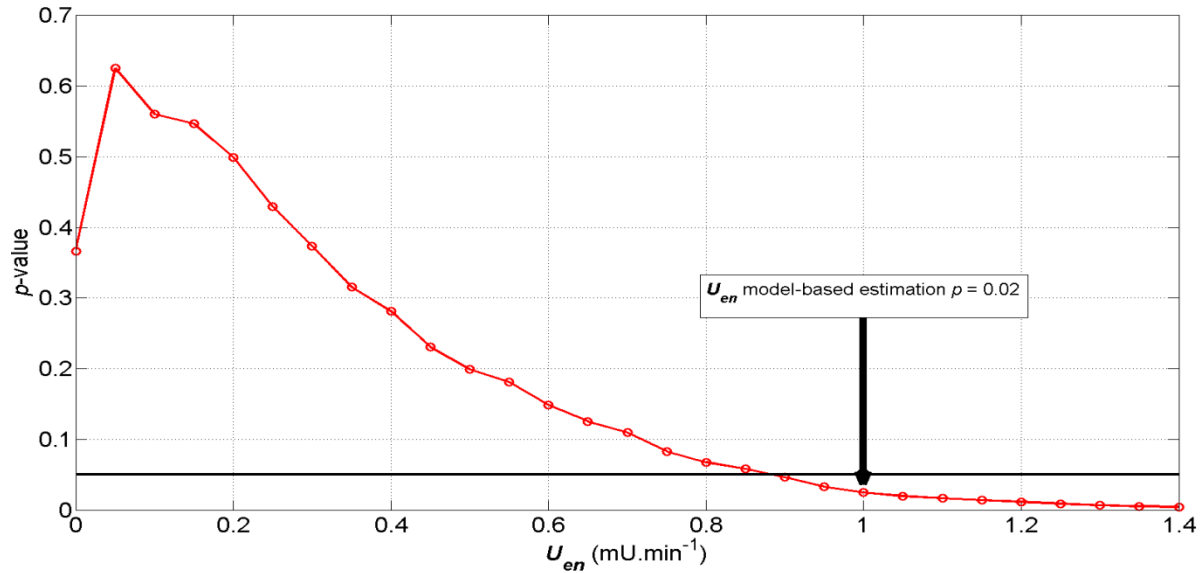


Figure 7.5 Endogenous insulin secretion corresponds to p -values that cause ΔS_I (\downarrow) of -29% at $t=2$ hours for the OFF/ON transition.

Plasma insulin levels also depend on endogenous insulin secretion. Physiologically, U_{en} is determined by glycaemic level and the ability of β -cells to respond to blood glucose concentrations and changes. However, it was suggested that an increase in endogenous insulin secretion may occur in response to HD treatment with PS membrane dialyzer due to reductions in plasma insulin (Abe *et al.* 2007; Jaber *et al.* 2000; Liljenquist *et al.* 1978). As the p -value of U_{en} does not reach $p=1$, it is assumed that variance in U_{en} could not contribute to the observation

ΔS_I . In particular, the PS membrane can reduce plasma insulin significantly in HD (Abe *et al.* 2007; Abe *et al.* 2011). Thus, S_I would also be expected to decrease with an unmodeled increase in U_{en} during the initial period of HD treatment to maintain G level.

7.4 Limitations

Model-based S_I is an indication of overall glucose metabolism of critically ill patients and does not necessarily reflect the precise cellular physiology of peripheral insulin sensitivity. The larger model-based ΔS_I at a cohort level found in this study are unlikely to be caused by actual variance in true peripheral S_I at a cellular level. In particular, there is no apparent stimulus induced by haemodialysis to directly affect S_I . Thus, ΔS_I reflects changes in renal clearance or/and endogenous insulin secretion, which result in changes in the modeled S_I calculated based on fixed assumptions for these values.

In particular, the ICING-2 model prediction of U_{en} is made in terms of blood glucose concentration in the absence of direct measurement of C-peptide (Pretty 2012). Hence, the effect of dialysis on U_{en} cannot be defined patient-specifically by the model without added data that was not available in this study. It is also reported that endogenous insulin secretion is also affected by exogenous insulin (Argoud *et al.* 1987; Liljenquist *et al.* 1978). As plasma insulin levels are suspected to decrease during dialysis, it may also be suspected that endogenous insulin secretion would increase, at a cohort level. An increase in the modeled S_I over time should be observed at the OFF/ON transition, contrary to the observations here, which were much smaller. Hence, U_{en} dependence on blood glucose concentration would confound the observed effect and can be discounted as a contributor to the shifts in the modeled S_I in this study. Thus, it is most likely that effective renal clearance increases during HD and decreases after HD treatment, and is the dominant factor.

Effective U_{en} identification cannot be undertaken with only glucose data (Docherty 2011; Docherty *et al.* 2009). Thus, a direct measurement of C-peptide should be included in future for direct quantification of the effects contributing to the glycaemia of ARF patients. The results of this study could be used to confirm these results to power a further study that segregates these potential contributing effects.

All critically ill patients with ARF in this study were undergoing SPRINT tight glycaemic control (TGC), where the minimal changes in G illustrated that SPRINT was successful in controlling glycaemia during these transitions. Glycaemic levels and the tightness of this protocol also to ensure the analysis results are not biased by variations in glucose levels which can affect stress response and thus S_I levels. Although there were two diagnosed type 1 diabetes patients in this cohort, almost no bias in ΔG was observed. Thus, the confounding factor plays no role.

7.5 Summary

Overall, this investigation suggests that the most likely contributor to the observed changes in S_I was the high HD insulin clearance, which was modeled by the renal insulin clearance term. The effect of HD on plasma insulin and the mechanism of insulin clearance among critically ill patients with ARF were shown in this study to be a contributor in overall effective S_I , which determines glycaemic concentration. However, further in-depth study must be undertaken to measure the specific effects of HD. A prospective cohort and clinical studies with direct insulin and C-peptide assays on this cohort may deliver better understanding in insulin kinetics during HD treatment. A broad comparison from a different cohort of varied HD duration with mixed levels of insulin resistance will also clarify the effects of ΔS_I , revealing further details in the underlying contributors of specific insulin resistance.

The distinct change in model-based insulin sensitivity during HD treatment was a significant and observable aspect of critically ill patient physiology. The findings were consistent with the presence of effects of HD treatment in a majority of ARF patients from other studies. Clinically, the effect of the main contributors (n_K and U_{en}) of effective insulin sensitivity changes during HD from a baseline model or clinical assumptions suitable for other patients should also be considered in glycaemic control. However, the precise pharmaco-kinetics/dynamics driving this change remain ambiguous. These results justify larger cohort investigation with specific measurement of insulin secretion and renal clearance to differentiate these impacts.

Chapter 8. Conclusion

Diabetes is an epidemic disease that yields catastrophic implications on quality of life, economic burden on healthcare systems and the population worldwide. In particular, both the total number of affected individuals and the level of associated complications is drastically growing. The major complications include polyneuropathy, blindness, kidney failure and limb amputations and are beginning to consume a major and increasing portion of worldwide healthcare costs.

One of the main pathological factors leading to type 2 diabetes is insulin resistance (*IR*), an impaired ability of the body to utilise the available insulin as blood glucose increased. *IR* is evident up to 10 years before type 2 diabetes is discovered. With early diagnosis of T2DM will give an opportunity to initiate appropriate treatment and lifestyle interventions to prevent and mitigate the effects of this disease before its spread and becomes chronic. Thus, several diagnosis assessments with various clinical settings have successfully been proposed in prior studies. However, the underlying causes that contribute to the erratic physiological effects in the model-based assessments are still unclear and ongoing.

A particular model-based approach allows the observation of metabolic states and defects with a minimally invasive test protocol. This minimal test is achieved by compensating for the lack of measured data with physiologically accurate models and parameters. A much more accurate and complete assessment of the metabolic system is thus possible, than is possible from surrogate metrics. A short clinical test has specifically developed by integrating practical clinical aspects and modelling techniques to further investigate the dynamics of the metabolic effects and its causes especially in IGT/T2DM.

Importantly, to validate the model-based S_I tests, parameter identification of PK-PD models using pilot DISST responses with iterative integral method (IIM) allows an independent examination of the model roles of α_G and n_I for describing true physiological glucose-insulin pharmacodynamics and pharmacokinetics. Increasing the number of identified participant-specific parameters did not necessarily improve model accuracy, as plasma insulin model fitting error was quite high compared to fitting error in plasma glucose. Very low values for α_G and n_I

are found to produce the most intra-patient variability in S_I . A model accounting for insulin receptor saturation of $\alpha_G=0.05$ with $n_I=0.055$ enhanced the repeatability in S_I . These S_I values derived using these α_G and n_I parameter values have correlation of $R=0.85$ with gold-standard EIC. This set of parameter values produced low median S_I variation amongst all subjects where the inter-quartile range was also tight with median 12% [IQR: 11, 14]%. A cohort of patients with mixed levels of insulin resistance will also further validate the accuracy of the model-based S_I and may provide a better understanding of the contributing factors of insulin resistance. A unique participant-specific identification of α_G and n_I might suggest the best possible repeatability of S_I . This might also increase model fitting accuracy of plasma insulin and interstitial insulin.

An analysis using the ICING model shows the distinct existence of an incretin effect as an observable aspect of critically ill patient physiology. The observation of this metabolic effect was based on ΔS_I at ON/OFF and OFF/ON enteral feeding transitions. The most significant observable incretin effect of decreased in $\Delta S_I=-36$ was found at $t=4$ hours after (ON/OFF) EN feeding was stopped. The findings were consistent with the presence of an EN-related incretin effect in a majority of critically ill patients. Clinically, the existence of this effect at EN nutrition transitions should also be considered in the management of glycaemia and could influence design of this therapy. While the results observed valid surrogates of the incretin effects, a prospective study with direct measurement and powered by these results may be required to confirm the outcomes directly.

Presumably, the incretin effects that occur during OGTT potentially adds] significant value to the DISST model via tracking and evaluating ϕ_D and ϕ_P controller gains of U_{en} control model amongst normal subject who underwent both DISST and OGTT challenges. However, insignificant results were observed which indicated that incretin effects were unobservable in OGTT response as $\frac{\phi_D}{\phi_P}$ OGTT < $\frac{\phi_D}{\phi_P}$ DISST. The U_{en} control model and methods proposed here enable one to obtain a specific observation on both β -cell function and insulin kinetics in a single approach and delivered a good compromise between model simplicity and accuracy. Hence measurements of plasma C-peptide concentration and the use of the independent glucose-insulin PK-PD models for glucose-insulin regulatory mechanism could provide unique insight into the

pathogenesis of T2DM and the metabolic syndromes. Finally, the proposed U_{en} control model of insulin secretion and kinetics will provide the ability to precisely define the pancreatic β -cell function in relation to insulin sensitivity.

An analysis of the impact of HD therapy on insulin sensitivity and insulin kinetics was also performed. The investigation suggested that the most likely contributor to the observed changes in S_I was the HD insulin clearance. The effect of HD on plasma insulin and the mechanism of insulin clearance among critically ill patients with ARF were shown in this analysis to be a contributor in the overall effective S_I , which physiologically illustrate the glucose-insulin metabolic system during HD therapy.

The distinct change in the model-based insulin sensitivity during HD treatment was a significant and observable aspect of critically ill patient physiology. The findings were consistent with the presence of effects of HD treatment in a majority of ARF patients from other studies. Clinically, the effect of the main contributors (n_K and U_{en}) of effective insulin sensitivity changes during HD from a baseline model or clinical assumptions suitable for other patients should also be considered in glycaemic control. However, the precise pharmaco-kinetics/dynamics driving this change remain ambiguous. These results justify larger cohort investigation with specific measurement of insulin secretion and renal clearance to differentiate these impacts. Further in-depth study must be undertaken to measure the specific effects of HD. A prospective cohort and clinical studies with direct insulin and C-peptide assays on this cohort may deliver better understanding in insulin kinetics during HD treatment. A broad comparison from a different cohort of varied HD duration with mixed levels of insulin resistance will also clarify the effects of ΔS_I , revealing further details in the underlying contributors of specific insulin resistance.

Chapter 9. Future Work

The analyses presented in this thesis addressed several limitations in assessing and integrating the dynamics and metabolic effects of PK-PD models in wider clinical and research settings. Further improvements in the clinically validated PK-PD models hopefully can provide better understanding of these underlying effects that cause variability within physiological range of PK-PD models.

9.1 Incretin measurements and modelling

The relative insulin secretion across PN/EN feeds must be directly measured to validate the observation of incretin effects in critically ill patients. The analysis in Chapter 5 only used EN nutrition without gastrointestinal hormones (GLP-1 and GIP) measurements. Thus, an ideal study design for the observation of incretin either in critically ill patients would also use PN feeding in a cross-over format.

A further study with varying insulin resistant levels cohort could also incorporate direct measurement of the gastrointestinal hormones. This approach would allow direct incorporation and identification of additional incretin hormone-related model parameters, as well as direct measurement of the effect without relying on PN measurements. Also, the variability of the incretin effects can be observed with a larger and varied insulin resistant cohort. Thus, modeling GLP-1/GIP with respect to insulin secretion and β -cell glucose sensitivity could validate the assumptions this evaluation of the incretin effect. This proposed approach will validate the prior finding as it will allow simultaneous estimation of both β -cell glucose sensitivity and the ability of GLP-1 and GIP to enhance insulin secretion.

9.2 Stomach and gut model/meal stimulator

An accurate model of the glucose-insulin system in the postprandial state is required for studying the pathophysiology of the diabetes. In particular, such a model would be required for design and evaluating the glucose sensor/control algorithms, decision support systems for treating diabetes and to investigate the metabolic effects in the postprandial state. The importance of developing a

stomach and gut model (meal simulator) is to allow model-based interpretation of the oral glucose ingestion used in everyday meals. However, the oral route is more difficult to model than the intravenous route because the model has also to describe the glucose ingestion and absorption processes. A few oral simulation models are available (Andreassen *et al.* 1994; Lehmann and Deutsch 1992). The major limitations of these models are that both have been only validated on plasma concentration data not in clinical setting.

A stomach and gut model should describe the physiological events that occur after a meal by uniquely integrating the validated stomach and gut model with glucose-insulin (PK-PD) models. This would direct the investigation of the metabolic effects (i.e. incretin effects) in the postprandial state that is assumed to contribute to the overall insulin secretion by β -cells in pancreas. Thus, a parameter identification study on the stomach and gut model that enhances insulin secretion via incretin effects should be undertaken.

9.3 Endogenous insulin secretion model

An endogenous insulin secretion control model that is a function of glucose concentrations has been proposed to segregate the incretin effects after oral glucose ingestion. Due to limited glucose data and unavailability of C-peptide measurement, the proposed model failed to segregate this metabolic effect between IV and oral glucose inputs. Although this U_{en} control model is well-functioned in DISST responses, the wide range of physiological disorders of *IR* individuals obscure the true dynamics and metabolic effects that occurred. A refined study of β -cell function that affects insulin secretion would combine higher resolution data to accurately define the secretion profile. Also, the blood glucose assays coinciding with the other samples (C-peptide and insulin) would provide a better basis for a model as a function of both G and C-peptide with its time derivatives.

9.4 Reliability and high frequency of clinical data measurements

It is important to measure high frequency of glucose and insulin data especially after IV/oral glucose input. The high data density may enable other model parameters to be identified, which portray the true physiological effects or at least, characterised in a more participant-specific

manner. Thus, a further study using current glucose meter devices with continuous glucose monitoring (CGM) sensor technology could potentially increase the data resolution. Current CGM technology in glucose meters consists useful features:

- Multi-test systems: Some systems use a cartridge or a disc containing multiple test strips. This has the advantage that the user does not have to load individual strips each time, which is convenient and can enable prompt testing.
- No coding systems: Older systems required coding of the strips to the meter. This carried a risk of miscoding, which can lead to inaccurate results/noise. Two approaches have resulted in systems that without coding. Some systems are auto-coded, where technology is used to code each strip to the glucose meter. And some are manufactured to a single code, thereby avoiding the risk of miscoding.
- The downloadable meters. Almost recent systems come with software that allows the user to download meter results to a computer. This information can then be used, together with clinicians' guidance to enhance and improve diabetes management. The meters usually require a connection cable, unless the meters are designed to work wirelessly with an insulin pump, or are designed to plug directly into the computer.

Overall, continuous monitoring allows examination on the blood glucose concentration response to insulin, physical exercise, food and other factors. The additional data can be useful for setting correct insulin dosing ratios for food intake and correction of hyperglycemia. Monitoring during periods when blood glucose concentrations are not typically checked (e.g. overnight) can help to identify problems in insulin dosing (such as basal levels for insulin pump users or long-acting insulin levels for patients taking injections). Monitors may also be equipped with alarms to alert patients of hyperglycemia or hypoglycemia so that a patient can take corrective action(s) (after fingerstick testing, if necessary) even in cases where they do not feel symptoms of either condition. While the technology has its limitations, studies have demonstrated that patients with continuous sensors experience less hyperglycaemia (Bode *et al.* 2004; Boland *et al.* 1999; Klonoff 2005; Pretty *et al.* 2010).

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